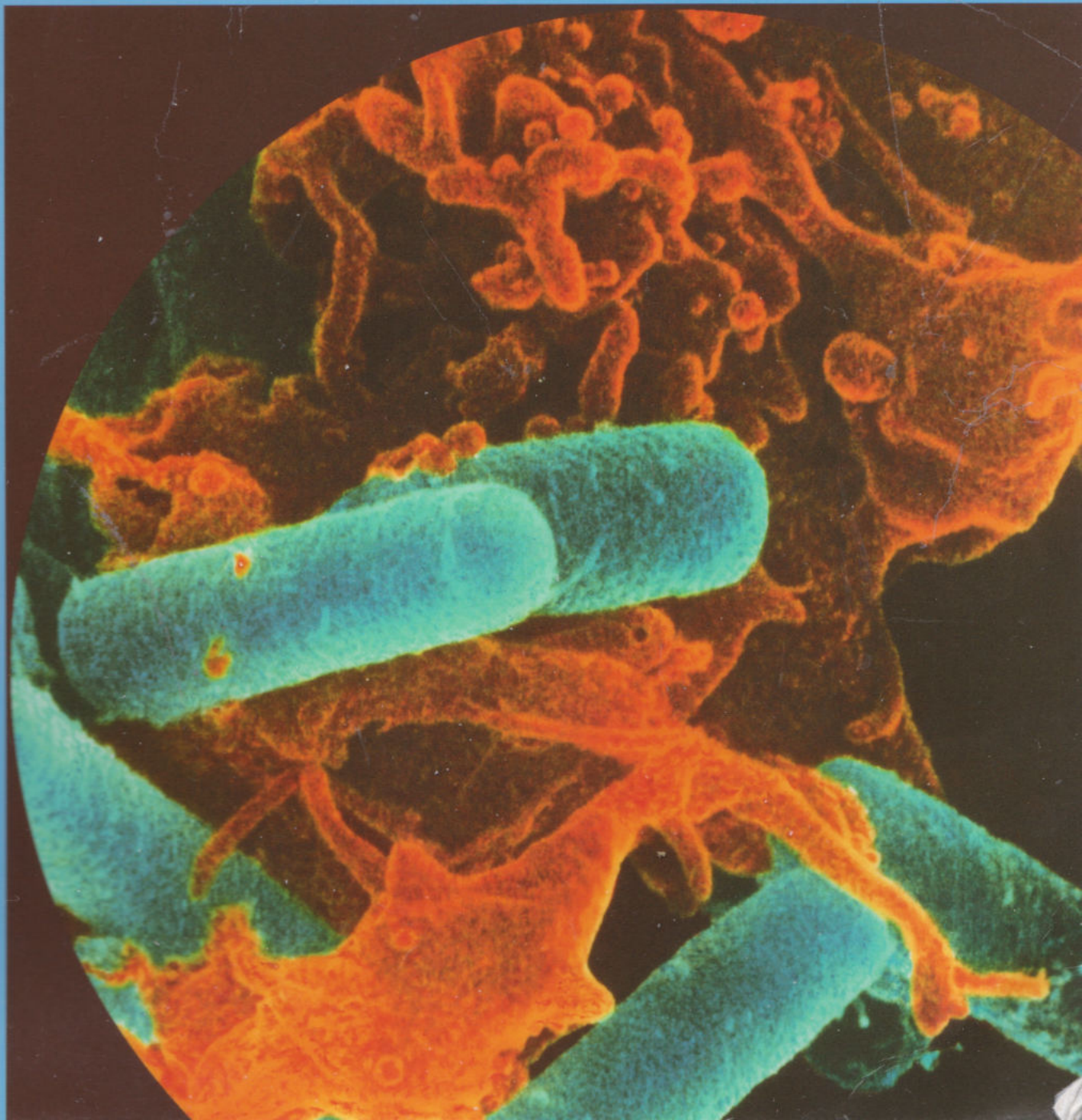


S320

INFECTIOUS DISEASE

3

IMMUNOLOGY



S320

INFECTIOUS DISEASE

3

IMMUNOLOGY

prepared for the Course Team by
David Male



Cover picture: Coloured scanning electron micrograph of a polymorphonuclear white blood cell, or leukocyte, attacking *Bacillus cereus* bacteria. The leukocyte (orange) is attaching to and engulfing the *Bacillus* cells (blue, rod-shaped). It uses enzymes to digest the bacteria and this process of engulfment and digestion is known as phagocytosis. Magnification x 25,000.

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CONTENTS

1	CELLS AND ORGANS OF THE IMMUNE SYSTEM	
1.1	Introduction to immunology	7
1.2	Cells of the immune system	14
1.3	Cell traffic (CD1)	26
	Summary of Chapter 1	26
	Learning outcomes for Chapter 1	27
2	IMMUNE DEFENCES AGAINST INFECTION	
2.1	Antibodies	29
2.2	Influenza mini-lecture (CD2)	38
2.3	Complement and inflammation	39
2.4	Antibodies and complement in defence against bacteria	48
2.5	Phagocytes and phagocytosis	51
2.6	The role of mononuclear phagocytes in bacterial infections	58
2.7	Overview of antibacterial defences	62
2.8	Eosinophil reactions to helminths	63
2.9	Cell-mediated immune responses	66
2.10	Antibody mediated immune responses	79
2.11	Immunological memory	88
2.12	Th1 and Th2 responses	90
2.13	Immune defences against viruses	94
2.14	Immunosuppression	102
	Learning outcomes for Chapter 2	104
3	HIV AND AIDS: A CASE STUDY	
3.1	Human Immunodeficiency Virus mini-lecture (CD2)	107
4	SECONDARY CONSEQUENCES OF INFECTION	
4.1	Tumour development	109
4.2	Hypersensitivity	111
4.3	Hypersensitivity and autoimmunity following infectious disease	113
	Summary of Chapter 4	114
	Learning outcomes for Chapter 4	114

5	ADAPTATIONS OF THE HOST AND PATHOGEN POPULATIONS	
5.1	Genetics of susceptibility to infectious disease	115
5.2	Strategies of immune evasion	123
5.3	Evolving hosts: evolving infections	132
	Summary of Chapter 5	133
	Learning outcomes for Chapter 5	133
	SUMMARY OF BOOK 3	134
	REFERENCES AND FURTHER SOURCES	136
	ANSWERS TO QUESTIONS	137
	ACKNOWLEDGEMENTS	142
	INDEX	144

1 CELLS AND ORGANS OF THE IMMUNE SYSTEM

1.1 Introduction to immunology

This book focuses on the human immune system and its responses to infection. The text accompanies *Immunology Interactive*, which consist of two CD-ROMs. The animations and associated material on CD1 illustrate, summarize and consolidate the text. CD2 contains a series of mini-lectures which show how the immune system works in particular diseases. Each visit to the CD-ROMs will require about one hour of study time. *Immunology Interactive* contains far more material than is required for this course. However, you may well find the additional material useful for your project work, or just as interesting background information on the immune system. Within this book, any reference to CDs means the CDs of *Immunology Interactive*. One valuable function of *Immunology Interactive* is that it serves as an illustrated glossary. The background expansion screens on CD1, accessible from the alphabetical index via the information button (i), are a huge source of immunological information. If you encounter terms you do not understand, make a note and use this resource the next time you are working with your computer.

The immune system has evolved to protect us against infection. So in order to understand how infections develop in an individual, we must now look at the different mechanisms that the body deploys in its own defence. The immune system is often thought to be one of the most complex systems of the body, mostly because there are so many different ways in which it can react against pathogens. The reason for the complexity of the immune system is, however, easy to understand; infectious agents vary enormously in size, life-style, location within the body and in the damage which they produce, so different types of immune response are appropriate for each group of pathogens. The importance of the immune system is clearly seen in individuals who suffer from immune deficiency. In these circumstances the person becomes much more susceptible to infection. Sometimes this can be a general increase in susceptibility. For example, children with rare genetic defects who are born with non-functional lymphocytes, are open to infection with a wide range of bacteria, viruses and parasites. In other cases selective damage to a part of the system (for example in AIDS) leads to susceptibility to a more restricted group of pathogens.

As we consider how the immune system combats infection in an individual, we must not lose sight of the broader picture. Over the centuries, immune systems have exerted a selective pressure on different pathogens, which in turn, have found ways of countering or evading the immune defences of their host. So a dynamic balance has evolved between humans and the pathogens which infect them. Certainly, the immune system has shaped the evolution of pathogens, but by the same token, our immune systems have been shaped over millions of years by the infectious agents that we have encountered. You will learn more about the coevolution of hosts and pathogens in Book 5, *Evolving Infections*.

Immunology is a major subject in its own right, and there is insufficient space within this course to explore it fully. So we will concentrate on the ways in which the immune system tackles different pathogens, and will leave aside much of the fascinating molecular and cellular biology which underlies these responses. This book starts by introducing the cells of the immune system and then considers how they interact, firstly to recognize an infectious agent, and then to eliminate it or contain it. Different diseases will be introduced along the way to illustrate the different types of immune reaction which are directed against each group of pathogens.

1.1.1 The immune system

What are the major components of the immune system? The principle cells of the immune system are **leukocytes** or white blood cells, which are distributed throughout all the other organ systems of the body, and are also located in specialized lymphoid organs. You will find that immunologists refer to two types of lymphoid tissues: the **primary lymphoid tissues** are the bone marrow and thymus, where the cells of the immune system are first produced and trained; the **secondary lymphoid tissues** include the spleen, lymph nodes, mucosa-associated lymphoid tissues (MALT) including Peyer's patches (Figure 1.1).

Lymphoid tissues refers to all the collections of lymphocytes found in the body, whereas the term 'lymphoid organs' is generally reserved for discrete encapsulated tissue, such as lymph nodes.

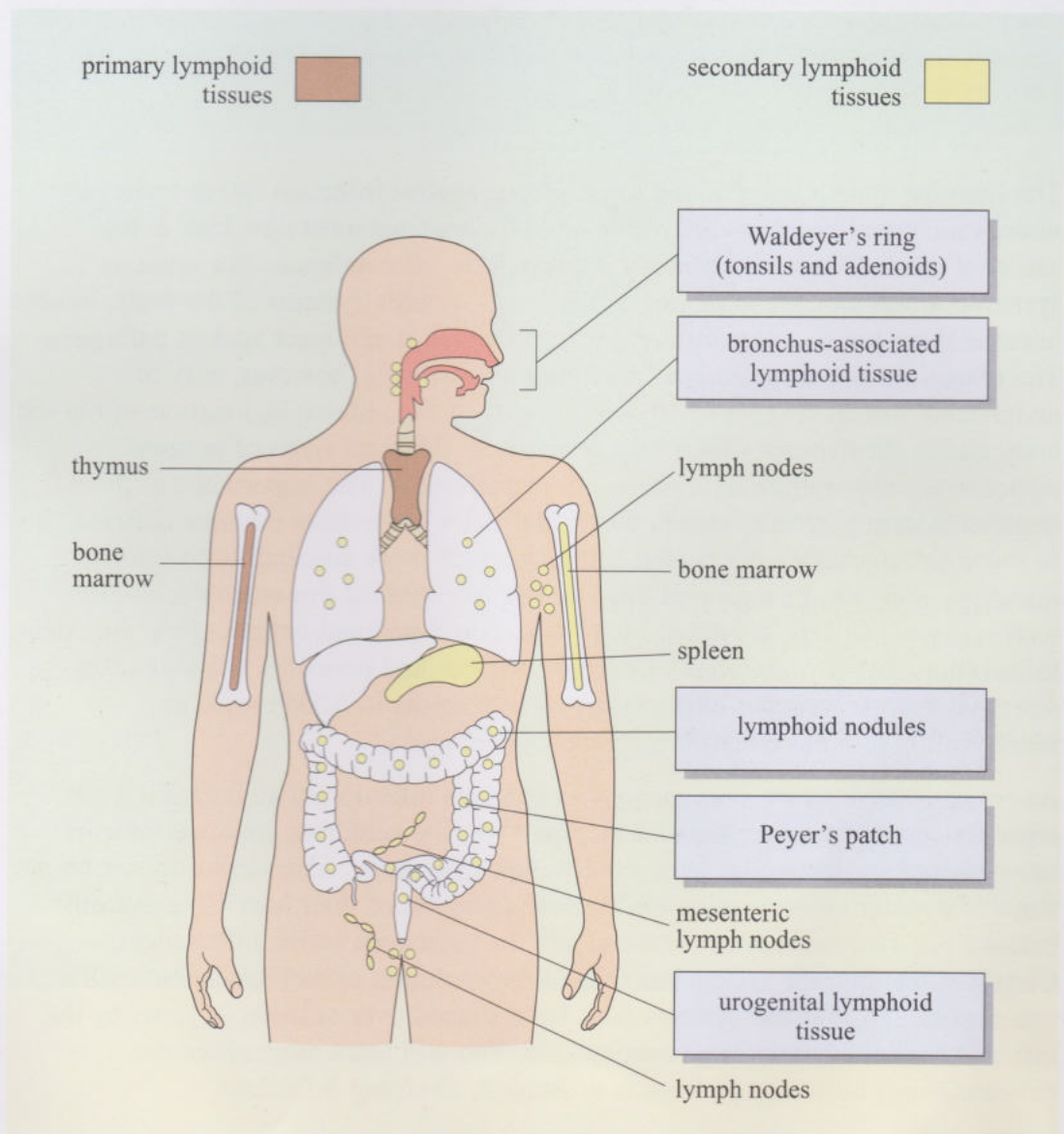


FIGURE 1.1
The primary lymphoid tissues are the bone marrow and thymus, where lymphocytes undergo their initial differentiation and development. The secondary lymphoid tissues include the lymph nodes and spleen, and the collections of mucosa-associated lymphoid tissues (MALT) indicated by the boxes. These tissues contain mature lymphocytes that can respond to infection. Since many pathogens enter the body through mucosal surfaces, the MALT is particularly important, in protecting these potential sites of entry. Note that bone marrow contains mature cells and can therefore also be considered a secondary lymphoid tissue.

These tissues contain the leukocytes that will initiate the immune response. There are numerous different types of leukocyte, but two major groups of cells are the **lymphocytes**, which are responsible for recognising foreign material, and **phagocytes** whose main function is to internalize it and destroy it. Taken together, these cells constitute a major organ system of the body. To give you some idea, the bone marrow in an adult produces about eight million new phagocytes every minute. These are released into the circulation in order to patrol other tissues. Cells of the immune system are highly mobile within the body. They move between the lymphoid organs and other tissues of the body, using the blood and lymphatics as routes of migration (Figure 1.2). This means that the population of leukocytes which is seen in the blood is just that group of cells which is in transit from one place to another, and they constitute a tiny proportion of the whole. Infection can affect any organ of the body and infectious agents can potentially enter the body by a number of routes. For this reason, lymphoid organs are strategically sited throughout the body to intercept the spread of infection, and leukocytes migrate through all the other tissues of the body to control any infection which has become established.

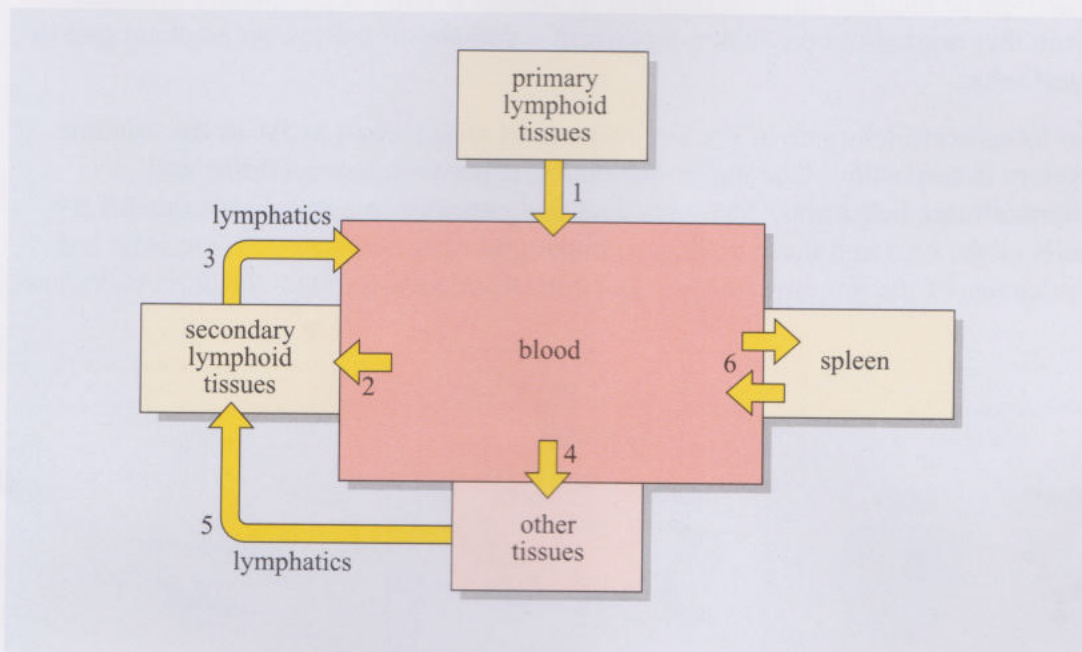


FIGURE 1.2 Leukocytes migrate around the body via the blood and lymphatic system. Lymphocytes leave the primary lymphoid tissues (1) enter the blood and colonize the secondary lymphoid tissues (2), by crossing specialized endothelial cells that line the blood vessels in these tissues. From there they can return to the blood via the lymphatics (3). (The lymphatic system is a network of blind-ended vessels that permeate all tissues except the brain.) Lymphocytes which have been activated following an encounter with antigen migrate more frequently through other tissues of the body (4), but can return from there to the lymphoid tissues via lymphatics (5). Lymphocytes can also migrate into and out of the spleen (6). Mononuclear phagocytes migrate from the primary lymphoid tissues to blood and then to other tissues, but can also recirculate via the lymphatics (1, 4, 5, 3). The other principal type of phagocyte, the neutrophil, makes a one way journey from the bone marrow to the tissues (1, 4).

1.1.2 Phases of the immune response

We often distinguish two principal phases of an immune response:

- 1 the recognition of the infectious agent; and
- 2 the effector phase of the response, when the immune system aims to eliminate the infection, or at least contain it and minimize the damage it causes.

Exactly how the body responds depends on which infectious agent is involved, and numerous other factors, such as the genetic make-up of the individual, the route of entry into the body and whether this is the first time the pathogen has been encountered.

1.1.3 How the immune system responds to different infections

You have already come across the enormous range of pathogens, which vary from sub-microscopic viruses a few nanometres in diameter which replicate inside cells, to tape-worms located in the gut that can be more than a metre long (Figure 1.3). The type of immune defence required to combat a virus is completely different from that needed to deal with a tapeworm – you would not use an elephant gun to hunt mice.

So let us consider some of the key features of an infection as far as the immune system is concerned. One major distinction is between **intracellular** and **extracellular infections**. Many bacteria and parasites live and divide *outside* the cells of the host and these pathogens come into direct contact with the cells and molecules of the immune system. By contrast, all viruses, many bacteria and some

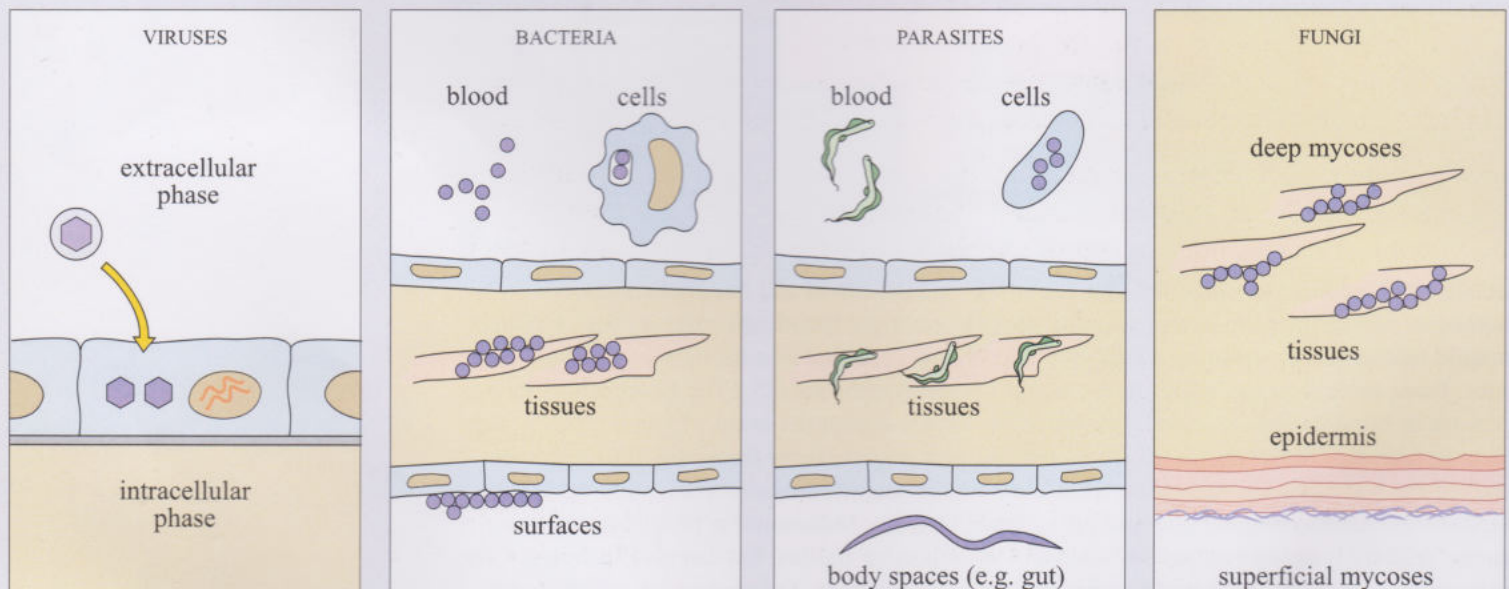


FIGURE 1.3 The immune system has to combat a great diversity of pathogens in different locations. Viruses have an intracellular and extracellular phase to their lifecycle and different immune defences are relevant to each phase. Bacteria may live in the blood (septicaemia) or within cells (e.g. tuberculosis) or within tissues (e.g. staphylococci) or on surfaces (e.g. cholera in the gut). Parasites also may live inside or outside cells, and many have complex lifecycles involving both phases. Parasitic worms can live in tissues (e.g. schistosomes) while large parasitic worms can occupy spaces such as the gut. Fungi are broadly divisible into superficial (surface) infections and deep mycoses that occupy tissues.

parasites live and replicate *inside* cells of the host. The type of response that develops against an intracellular pathogen is quite different from the response to an extracellular pathogen. In this case, the cells of the immune system recognize, not the infectious agent *per se*, but the infected cell of the body. Of course, to reach the host cell, intracellular pathogens have to travel through the blood or tissue fluids, and at this time they are susceptible to the immune defences which recognize extracellular pathogens. This characteristic, where different stages of infection are controlled by different immune defences, is typical of very many infections.

- ☐ From your knowledge of malaria (Book 1, Chapter 3), what stages of the life-cycle would you expect to be recognized by antibodies that protect against extracellular infections and which stages would be susceptible to lymphocytes that recognize infected host cells?
- ☒ Antibodies can recognize the sporozoite as it passes through the blood to the liver cell following first infection and later on different antibodies act against the merozoites as they move between red blood cells. Lymphocytes recognize infected liver cells and, in some circumstances, the infected red blood cells.

1.1.4 Routes of infection

Despite the differences between pathogens, there are only a limited number of routes by which infection can enter the body. Some infections remain superficial, (e.g. the fungi which cause athlete's foot) and normal intact skin is a very effective barrier against infection, since very few organisms are able to penetrate it. If, however, the skin is breached by a wound or a burn, the underlying tissues may become infected. Bacterial infections of wounds can be very serious, for example, hospital-acquired infections following surgery. Viruses too can enter the body following damage to the skin. For example the rabies virus carried in saliva enters the body at wounds caused by the bite of a rabid animal. It does not require a major wound to allow infection to enter the body across the skin. Splinters can cause infection with the bacteria that cause tetanus, and the bite of an infected insect is sufficient to cause the transfer of diseases such as malaria or yellow fever virus.

A major route for infection is across the mucous membranes of the respiratory tract, the gut and the genitourinary tracts. These membranes have a variety of unspecialized mechanisms that reduce the chances of pathogens attaching to them. These include the production of mucus, which traps many pathogens, and cilia on the surface of the cells that move the mucus up the bronchial tree to where it can be swallowed. Acid in the stomach and enzymes in the gut also have anti-microbial actions. The local bacterial fauna affords another important method of non-specific protection for these sites. The gut and vagina have their own normal collection of harmless microbes, which can compete with pathogens, so in some cases disease may develop if the normal balance of bacteria and yeasts at these sites is disturbed. Taken together, the non-specific barriers to infection, illustrated in Figure 1.4, are essential adjuncts to specific immune responses.

Many pathogens that enter the body do not cross the mucous membranes into the tissues. For example, the parasitic worms that infest the gut, enter the body by ingestion, and live in the intestine, but as they are lying within the gut they are still effectively outside the tissues of the body. Bacteria such as cholera produce devastating damage, but they too do not enter the tissues, and they produce their damage while attached to the cells lining the gut.

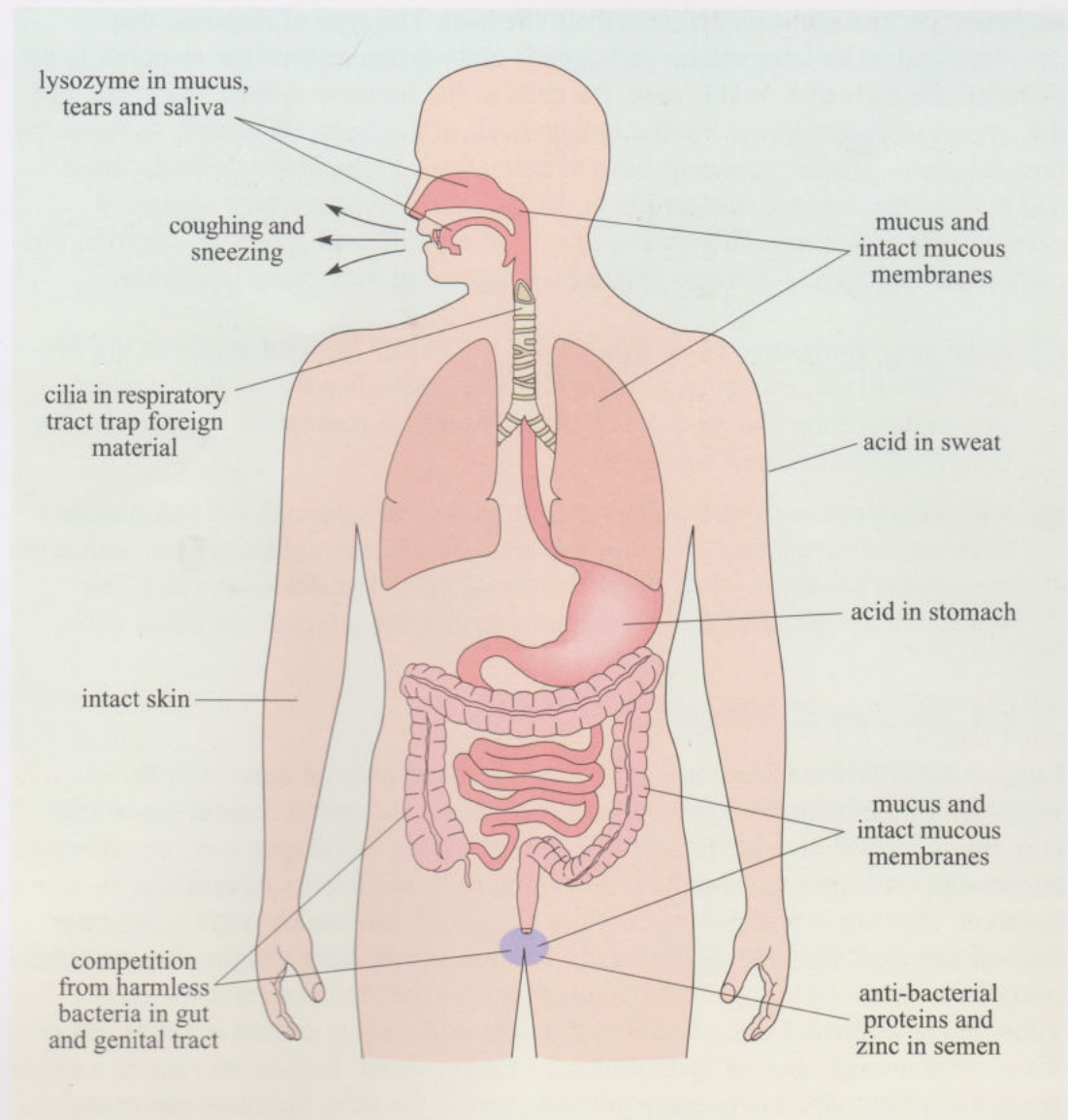


FIGURE 1.4 Physical and chemical barriers are an important element in host defence. It is only if these barriers are breached, that specific immune defences come into play.

Many pathogens however do get into the body. The infection may be limited to the immediate point of entry. For example, *Neisseria gonorrhoeae* which cause the sexually-transmitted infection gonorrhoea, first attach to the cells which line the urethra and then enter and infect just these cells. Other pathogens distribute more widely, and may move through the blood and tissue fluids, either in suspension or within cells they have infected. We refer to these as **systemic infections**. Once inside the body such infections will usually provoke a strong immune response, which develops within the lymphoid tissues.

- ☐ Thinking about the major routes of infection; where in the body would you expect most lymphocytes to be located?
- ☒ Most of the body's lymphocytes are located in lymphoid organs that are associated with the mucous membranes of the respiratory, gastro-intestinal and genitourinary tracts.

1.1.5 Secondary lymphoid tissues

Mucosa-associated lymphoid tissues (MALT)

Because the mucous membranes are such important points of entry for pathogens, these sites have large concentrations of specialized lymphoid tissues to protect them. You will probably be familiar with tonsils, which are lymphoid organs found on the sides of the throat, and perhaps also know of the adenoids that lie in the nose. These are both examples of **mucosa-associated lymphoid tissues (MALT)**. The respiratory tract has its own collections of lymphoid cells called **bronchus-associated lymphoid tissue (BALT)** and there are other specialized lymphoid tissues in the gut (**Peyer's patches**), the genitourinary tract and the eye. This is the first line of defence of the immune system – the place where pathogens first come into contact with lymphocytes which can recognize them. Later, in the effector phase of the immune response, lymphocytes in the sub-mucosal tissues produce a particular type of antibody that is released into the mucous secretions to combat the infection. Most potential pathogens never get beyond these first lines of defence.

Lymph nodes

Lymph nodes are small organs varying in size from a few mm to 1–2 cm. They contain collections of leukocytes and are distributed in different areas of the body. Lymph nodes are linked in chains by lymphatic ducts, so that the lymph flowing out of one lymph node via the *efferent* lymphatic, becomes the inflow to the next in line, via the *afferent* lymphatics. (Lymph is a fluid derived from the tissues that carries cells and foreign material to the lymph nodes.) Eventually, lymph returns to the blood stream via one of the body's two major lymphatic ducts. We sometimes think of the secondary lymphoid organs as guard-posts that are strategically placed to intercept any infectious agent that enters an area of the body. So for example the lymph nodes in the axilla of the arm will intercept infections which enter that part of the body. In this analogy, leukocytes are the troops of the immune system and they are able to move between the guard posts and into the hinterland of the tissues.

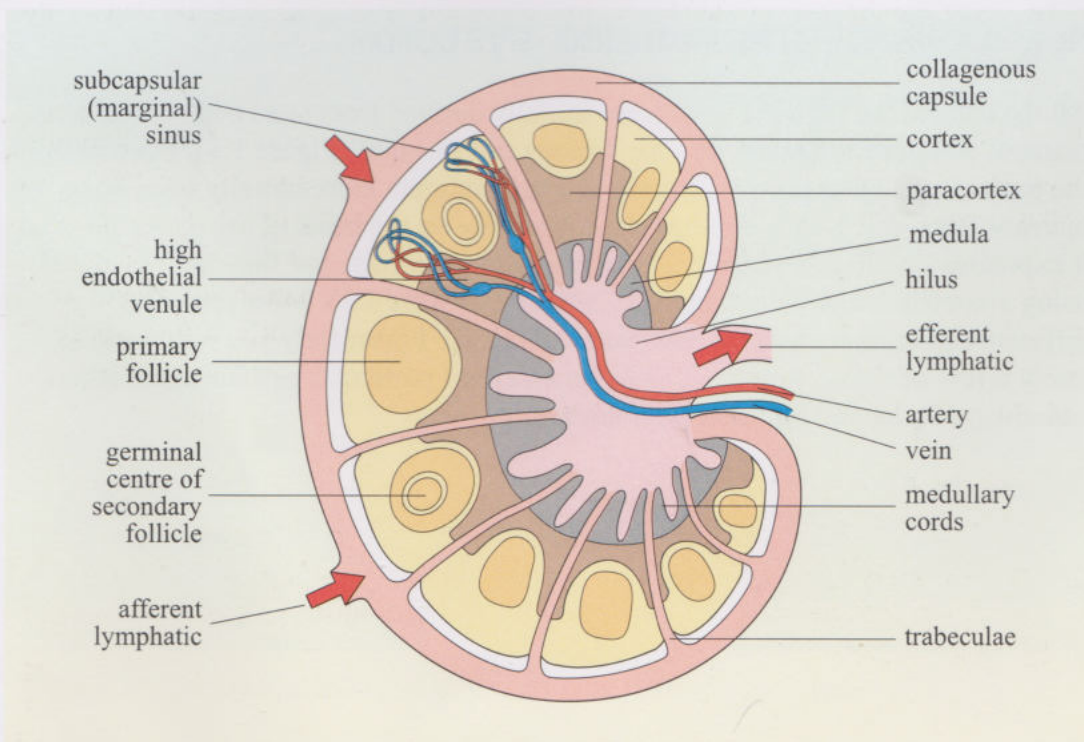


FIGURE 1.5

The structure of a lymph node. Cells and antigens enter the node through the afferent lymphatics and leave through the efferent lymphatic. Lymphocytes can also enter from the blood by migrating across the specialized high endothelial venules. Within the node, cells distribute to distinct zones. B cells proliferate and develop within the follicles of the cortex, while T cells are primarily located in the paracortex. The capsule, medullary cords and hilus are fixed structural elements of the tissue.

Lymph nodes have a well defined structure (Figure 1.5), with different sub-regions. Different types of leukocyte are localized within the regions, so that they can interact with each other appropriately to initiate and develop the immune response. Antigens and cells enter the node through afferent lymphatics, and cells and fluid leave through the efferent lymphatic. Cells can also enter the node from the blood as indicated in Figure 1.2.

Lymphocytes located in the local lymph nodes are responsible for the initial recognition of the infection and the development of the immune response. Once the immune response has developed, the cells will migrate out from the lymph node to the blood and ultimately cells will move to the site of infection to combat the pathogen there.

The spleen

The **spleen** is a large organ which lies just behind the stomach and it has a number of functions, one of which is as a lymphoid organ. In fact, the spleen contains two types of tissue called red pulp and white pulp and it is the white pulp that is a lymphoid tissue (the principle function of red pulp is to destroy effete red blood cells). The white pulp is distributed around the arterioles in the spleen and is often called the **peri-arteriolar lymphatic sheath (PALS)**. The key immunological function of the spleen is to take up infectious agents that may be circulating in the blood. The role of the spleen is made clear in people who have had their spleen removed surgically (to prevent internal bleeding after injury to the spleen). These people are much more susceptible to septicaemia, i.e. bacterial infection of the blood.

You will now realize that the cells of the immune system move between lymphoid organs and the other tissues of the body. The patterns of cell migration are complex and depend on the type of cell involved and whether it has been activated. We must now go on to look at the cells of the immune system in detail and examine their functions in an immune response.

1.2 Cells of the immune system

All the cells of the immune system are initially derived from stem cells in the bone marrow, which differentiate into the various cell lineages (Figure 1.6). Even cells in the thymus, the other primary lymphoid organ, migrate there initially from bone marrow. Each cell type is distinguished primarily on the basis of the molecules that it expresses on the cell surface. These are called markers, and they are organized using a nomenclature called the 'CD' system (see Box 1.1). More than 300 different cell surface markers have been identified. Fortunately you only need to know a few of these, which act as key markers of particular cell lineages; others can always be looked up in tables if needed.

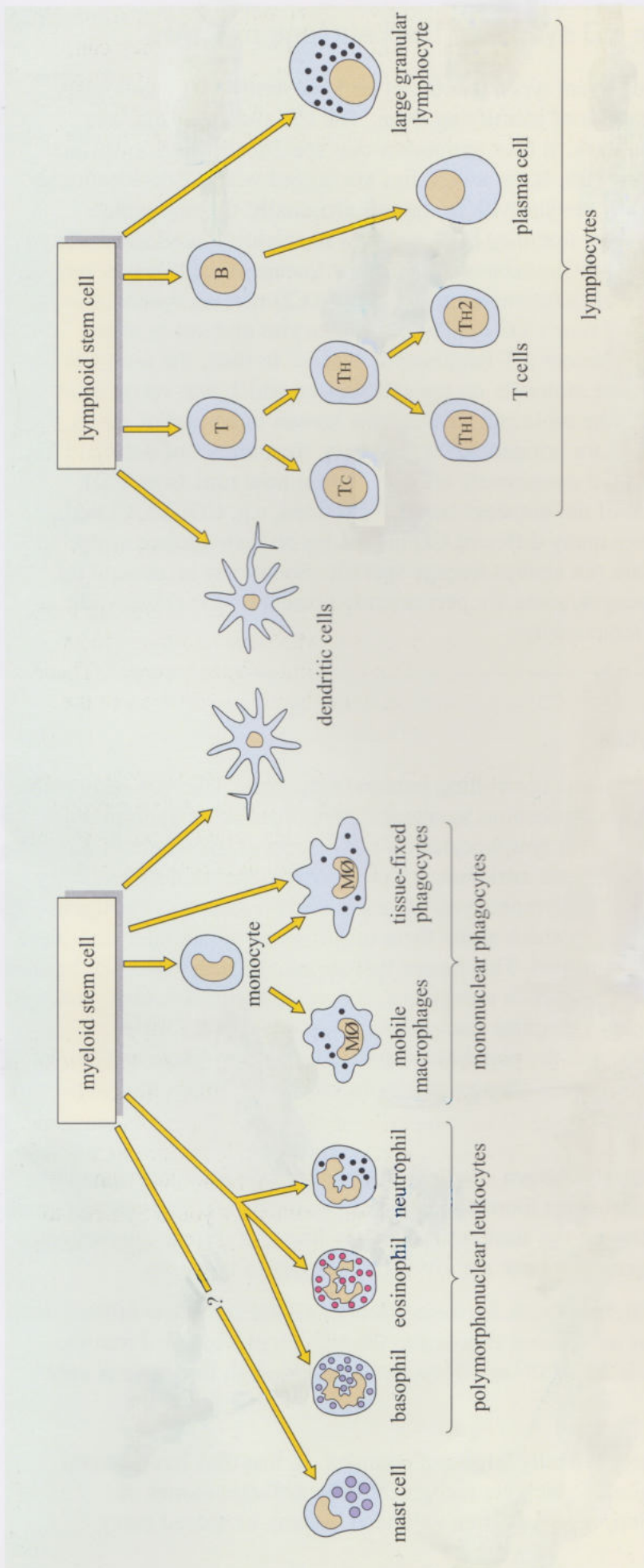


FIGURE 1.6 The principle groups of leukocytes involved in immune defence are all derived from stem cells in the bone marrow. Lymphocytes are either 'T cells' (T) or 'B cells' (B). T cells differentiate into helper (Th) and cytotoxic cells (Tc). T helper cells further differentiate into Th1 and Th2 populations.

Box 1.1 The CD system of cell surface markers

Since many of the different types of leukocytes look similar, immunologists had to find reliable ways of identifying them. The CD system of markers developed from this work. It uses antibodies that specifically bind to surface molecules on lymphocytes. If the antibodies are tagged with a fluorescent dye then cells that have the marker will be stained. Originally, the researchers would submit antibodies that they had produced to scientific workshops for evaluation. If an antibody bound to one particular lineage of cells it was said to identify a 'Cluster of Differentiation', hence the CD naming system. For example, antibodies of type CD3 bind to T-lymphocytes and not to other leukocytes. CD3 is therefore a T-lymphocyte marker. In time, the term CD3 came to be used for the molecule on the cell surface which was recognized by the antibodies. So the molecule CD3 is now known to be part of the T-lymphocyte's receptor for antigen. Over the years, the number of defined CD molecules has expanded enormously and the system now runs from CD1 – CD247, with several of the numbers being subdivided, e.g. CD62E, CD62L, CD62P. All cells have many different CD molecules on their surface at any one time. Markers are not always lineage specific. Some may be present on several different lineages, some are permanently present on the cells, while others only appear temporarily.

1.2.1 Lymphocytes

Lymphocytes are the key cells controlling immune responses. They are responsible for the initial recognition of infectious agents and they organize an appropriate response against them. In fact lymphocytes do not recognize the whole of an infectious agent, they recognize **antigens**, which are individual molecules or fragments of molecules. The lymphocytes have particular receptors – **antigen receptors** – on their surface which allow them to recognize the antigen, and these receptors are clonally distributed. This means that an individual lymphocyte has just one type of antigen receptor, which recognizes just one antigen. However, although each lymphocyte can only recognize one antigen, there are roughly 10^{12} lymphocytes in the body, and the population as a whole can recognize millions of different antigens – enough to recognize any molecule that the microbial world can throw at the immune system.

- ☐ If you consider a single antigen – say the haemagglutinin from influenza virus – how many lymphocytes are there likely to be in an individual that can recognize it? Make an estimate on the assumption that the immune system can recognize 10^7 different antigens and there are 10^{12} lymphocytes in the body.
- ☒ The number is relatively small, between a few thousand and a few million in an individual who has never been exposed to the antigen previously. From the figures above this is $10^{12} / 10^7$ or 10^5 on average, although the range is very great.

Antigenic molecules are generally large and complex, so that they have several potential antigenic regions, which are recognizable by different clones of lymphocytes. In addition, each pathogen will have several, or indeed many, antigenic molecules.

Clonal selection

As the numbers of lymphocytes which recognize an infectious agent are relatively small, the first thing that the immune system must do when an infection occurs is to expand the number of cells available which are capable of responding to those antigens. This process is called **clonal selection**, because the antigen selects and activates those clones of cells that recognize it. When an antigen binds to the antigen receptors on a lymphocyte and provided the cell receives appropriate additional signals, it will be driven to divide. Lymphocyte division within a lymph node can occur every 6 hours, an extraordinarily high rate of cell division. And it needs to be, because a race is now on between the lymphocytes and the infection. Bacteria can divide every 20 minutes while eukaryotic cells typically divide every 20 hours. It is essential therefore for the immune system to generate as many specific lymphocytes as possible in the shortest time, in order to mount an adequate immune response.

One consequence of the considerations above is that most lymphocytes cannot recognize the ongoing infection. Indeed most lymphocytes do not recognize any particular infectious agent, and will never be called upon during a lifetime to engage in an immune response.

- ☐ What is the point of having millions of lymphocytes present in the body which, although they can recognize antigens, cannot recognize antigens from any known infectious agent?
- ☒ The immune system does not know what it will encounter during a lifetime. We must have lymphocytes available which can recognize any foreign molecule. If we only had cells available that could recognize extant pathogens, then when pathogens mutate, we would have no cells available to recognize them and would thus be wide open to infection.

T cells and B cells

Let us now look in detail at the lymphocytes. They fall into two major groups – **T cells** and **B cells**. B cells develop in the **Bone** marrow, while T cells develop in the **Thymus** – the two primary lymphoid organs. As they develop, each clone of B cells or T cells generates an antigen receptor and expresses it on the cell surface. Each clone of lymphocytes is able to produce a different receptor by a complex process that involves re-arrangement of the genes that encode the antigen receptor. Cells that fail to make an antigen receptor because they have been unsuccessful in rearranging their genes, die. At this point the surviving cells undergo a process of **education**. Any cells that recognize self-molecules will either be deleted (eliminated), or turned off, so they cannot make an immune response subsequently. This leaves lymphocytes that are capable of recognising non-self molecules. These educated lymphocytes will now move from the primary lymphoid organs to the secondary lymphoid organs where they are ready to deal with infection. At this stage they have receptors which are capable of recognising an antigen, although they have not yet encountered any infectious agent. These cells are called naïve or virgin lymphocytes. Notice these two points about lymphocyte development:

- 1 Lymphocytes generate receptors *before* they encounter an antigen.
- 2 Cells that recognize self antigens are actually or functionally deleted.

These two processes are important aspects of immunology, but we cannot go into them here. If you would like to know more, have a look at the animations on 'Production of antigen receptors' and 'T cell education', which you will find on *Immunology Interactive* disk 1 (CD1). There is also some information on antigen receptors in SK220 Book 2, Chapter 5 and this is available on the *Reference CD-ROM*. This supplementary material will be best understood if you look at it when you have completed the immunology section, and only if you have time available.

How T cells and B cells recognize antigen

The antigen receptors on B cells and T cells are quite distinct. You can see the structures of these receptors in Figure 1.7. The **B cell receptor (BCR)** for antigen is a cell surface form of antibody or immunoglobulin which consists of two identical heavy chains and two identical light chains, so it is bilaterally symmetrical. The T cell antigen receptor is unsurprisingly called the **T cell receptor (TCR)** and it consists of two chains of about equal size called α and β on most T cells. Both of these receptors are associated with a set of molecules that signal to the inside of the cell if the receptor binds to its antigen. So for example the **CD3** molecule that you encountered as a marker of T cells, is actually the part of the receptor complex which transduces the activation signal to the cell.

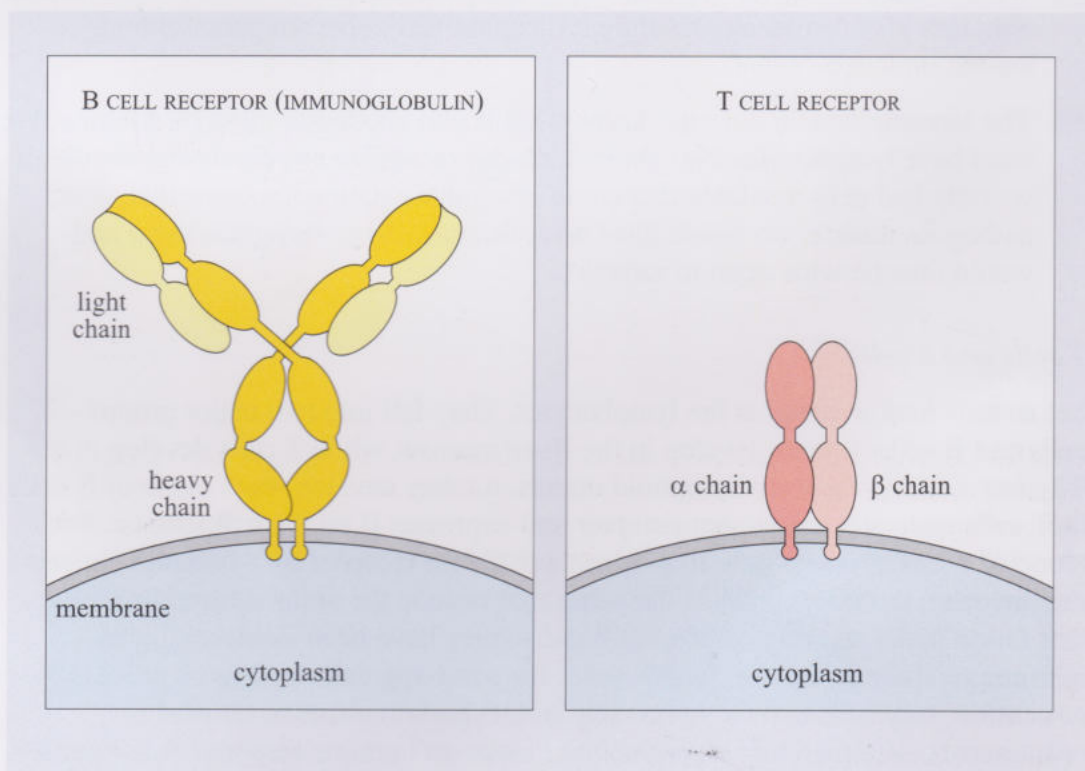


FIGURE 1.7 The structures of the antigen receptors found on B cells and T cells. The individual chains are folded into domains. There are two domains in the light chain and the TCR α and β chains and four or five in the immunoglobulin heavy chains, depending on the class of immunoglobulin involved. The receptors are associated with signalling molecules (CD3 on T cells and CD79 on B cells) which are not shown here.

It is absolutely essential to understand that B cells and T cells recognize antigens in different ways. B cells use surface bound antibodies as their receptor for antigen. Antibodies recognize molecular shapes called **epitopes** that are present on *intact*

molecules. In contrast, the T cell receptor recognizes *fragments* of antigens that are presented on the surface of other cells of the body. The fragments are presented by a group of molecules that are encoded within a region of the genome called the major histocompatibility complex, and so are called **MHC molecules**. The function of these molecules is described as **antigen presentation**. The ways in which T and B cells recognize antigen is shown in Figure 1.8. Every cell in the body samples its own internal proteins and places fragments of them on the cell. If a cell has become infected, molecules of the pathogen will be placed on the cell surface and can be recognized by T cells. And this is the important point: T cells are responsible for recognising if a cell has become infected.

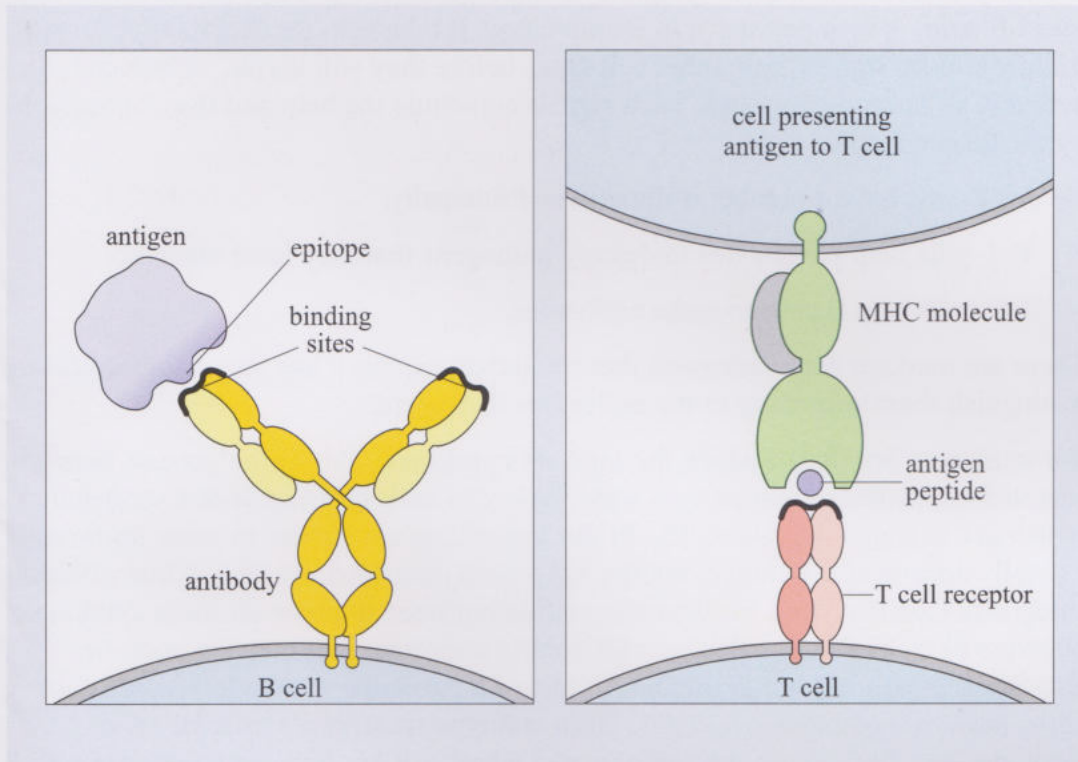


FIGURE 1.8 B cells and T cells recognize antigen in fundamentally different ways. Antibody (immunoglobulin) on the B cell has two antigen-binding sites that recognize an epitope (molecular shape) on the surface of an intact antigen. The receptor on T cells binds to an antigenic peptide (protein fragment) which is bound to a major histocompatibility complex (MHC) molecule, and it recognizes both the specific peptide and the MHC molecule. Thus T cells only recognize antigenic peptides located on the surface of other cells, which are using their MHC molecules to present antigenic peptides.

T cell populations

T cells are absolutely central to immune defence. In fact, there are several types of T cell each having different functions. The cells described above, which are responsible for recognising and destroying infected cells, are called **cytotoxic T cells** or **Tc cells**. The term **cytotoxicity** means being able to kill cells, and it is usually very desirable to kill a cell which has become infected.

- ☐ Can you think of any circumstances where it might *not* be desirable to kill an infected cell?

- Some cells of the body, such as nerve cells, are irreplaceable. If the immune system destroys a nerve cell, it has gone for good. For this reason, the immune response in the brain and spinal cord is limited. Nerve cells may remain chronically infected for years, for example in a herpes virus infection (cold sores). In this case the damage caused by the virus is moderate, and the alternative would be the destruction of the sensory nerve in which the virus has established its infection.

Tc cells are distinguished by the marker **CD8** and about one third of the T lymphocytes belong to this population. The remaining two thirds express the marker **CD4** and are called **helper T cells** or **Th cells**. Note that the markers are mutually exclusive, a mature lymphocyte has *either* CD4 *or* CD8 but not both. The idea of 'help' is very pervasive in immunology. It relates to the fact that leukocytes usually require signals from other cell types before they will divide, or become active in an immune response. Such signals constitute the help and this is provided by the helper T cells.

Helper T cells have a number of functions. Principally,

- Th1 cells help phagocytes to destroy pathogens that they have taken up;
- Th2 cells help B cells to make antibodies.

There are markers that distinguish these populations, but it has been more useful to distinguish them according to the molecules they secrete.

We must now briefly introduce the topic of **cytokines**. This is the generic term for secreted molecules produced by a wide variety of cells (not just leukocytes) and which act as signalling molecules. In this sense they are similar to some hormones. Literally dozens of different cytokines have been described, which fall into different categories (see Box 1.2). Fortunately, you do not need to know all these cytokines, but we will highlight ones that are particularly important in protective immune responses. If you need to know the actions of a particular cytokine, you can look it up in *Immunology Interactive* CD1. Each cell type secretes a particular set of cytokines that depends on the cell type and whether it has been activated. Only cells with specific receptors can respond to any particular cytokine and whether a cell has particular cytokine receptors depends on its type and on its state of activation.

Returning to the subject of T cell populations: Th1 cells produce a set of cytokines which allow them to interact with phagocytes, while Th2 cells produce cytokines which signal to B cells.

Antibody-producing cells

B cells are antibody-producing cells. We have already noted that they have antibody molecules on the cell surface which act as their antigen receptor. If a B cell binds to antigen and receives appropriate additional signals from Th2 cells it is driven to divide and differentiate, producing numerous **plasma cells**, a process which takes place primarily in the lymphoid tissues. Plasma cells produce a secreted form of antibody, which has an identical binding specificity to the surface antigen receptor on the original B cell from which the plasma cell was derived. It differs in that it lacks the portion of the molecule that keeps the B cell receptor bound to the cell membrane. Plasma cells are essentially antibody-producing factories. They live for a few weeks and then die. Their secreted antibodies are important components of the immune defence system.

Box 1.2 Cytokines and cytokine receptors

Cytokines are small proteins that act as signalling molecules. Because cytokines were originally identified by their activity, they were given a variety of appropriate names. For example, one cytokine that caused T cells to divide was called T cell growth factor. In time, it turned out that several different researchers could be working with the same molecule, but had given it different names because they had identified it as having different biological activities. It was therefore necessary to adopt a unified nomenclature. Immunologists now distinguish several families of cytokines listed below along with their abbreviations.

Cytokine family	Function
interleukins IL-1 to IL-26	involved in signalling between leukocytes
interferons IFN α , IFN β and IFN γ	involved in protection against intracellular pathogens
colony stimulating factors M-CSF, G-CSF, GM-CSF	promote the production of different populations of leukocyte
chemokines	more than 30 molecules involved in controlling cell migration and activation
tumour necrosis factors TNF α , TNF β	a group of molecules involved in inflammation
transforming growth factors	mediators of cell growth

Most of the cytokines have specific receptors so for example IL-3 binds only to the IL-3 receptor. But some cytokines bind to several receptors and some receptors bind several cytokines. This promiscuity of receptor binding is particularly prevalent with the chemokines. The expression of appropriate receptors determines whether a cell can respond to particular cytokines or not. Receptors are not always present on a cell but can be induced when a cell is activated, or at particular stages of its development.

- ☐ Most B cells and plasma cells die after a few weeks. Most T cells also only survive for a few weeks. Most phagocytes die within days of production, and this is all perfectly normal. Can you think of any reason why it is necessary to have such enormous levels of cell death in a normal immune system?
- ☒ Recall that phagocytes are produced at the rate of 10^8 per minute, and that clones of lymphocytes can divide every six hours during an immune response. In order that the immune system can respond to infection (i.e. adapt by producing more cells) but still remain a constant size, cell production is balanced by cell death over longer periods of time.

Large granular lymphocytes

Although the majority of lymphocytes in the body are B cells or T cells, 15% of the lymphocytes express neither a B cell receptor nor a T cell receptor. Over the years these cells have had many different names, but they are most often described

according to their appearance, as **large granular lymphocytes (LGLs)**. It is only recently that the function of these cells has become clearer. It had been noted that these cells were sometimes able to kill tumour cells or cells which had become infected with virus, i.e. they acted as cytotoxic cells (Figure 1.9). As they performed this function without appearing to use an antigen receptor they had been described as **natural killer (NK) cells**. We now know that these cells are an additional immune defence for recognising and dealing with infected cells. However, they use a different recognition system from cytotoxic T cells. You can think of cytotoxic T cells and natural killer cells as the police and the secret police. They both have the same function of protecting the body from subversion by viruses, but they go about it in different ways.

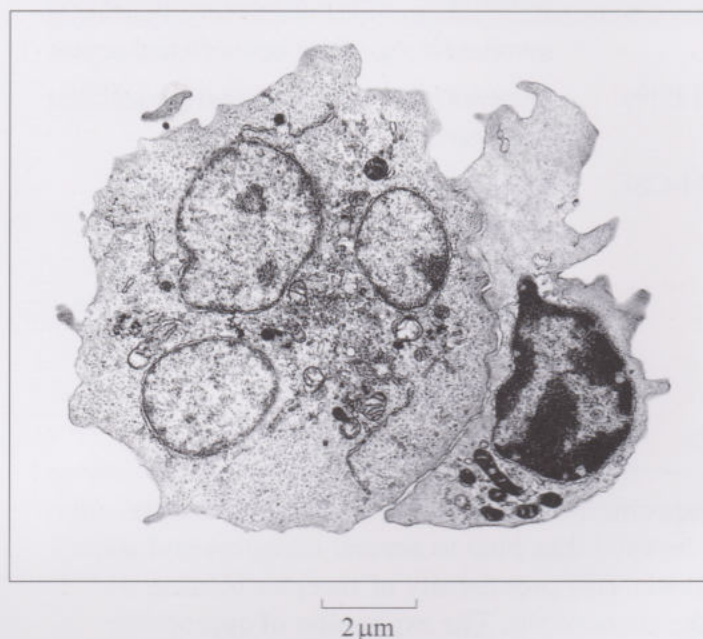


FIGURE 1.9

A large granular lymphocyte (right) is seen engaging a target cell in this transmission electron micrograph. The lymphocyte recognizes molecules on the surface of the target and can use a number of mechanisms to damage or kill it. A single lymphocyte can successively kill several target cells.

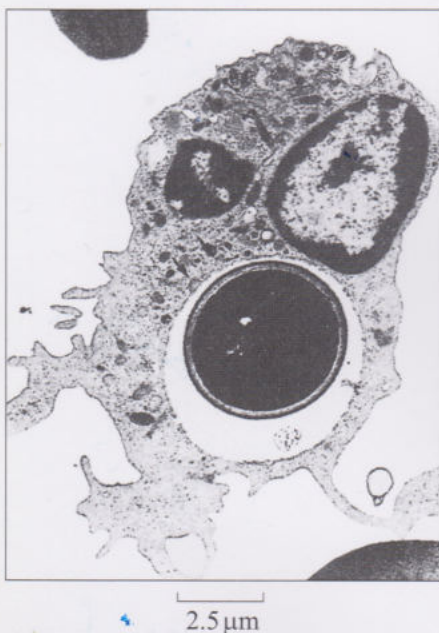


FIGURE 1.10

Electron micrograph of a neutrophil that has phagocytosed a yeast cell (*Candida albicans*). The yeast cell is enclosed within a membrane-bound vesicle called a phagosome. Lysosomal granules fuse with the phagosome causing their contents to be poured into the phagosome, and producing a phagolysosome. The yeast cell is broken down by enzymes acting within the phagolysosome.

1.2.2 Phagocytes

The process by which cells internalize material by incorporating it into intracellular vacuoles, is called **endocytosis**. If a cell takes up a large particle, such as a bacterium or yeast cell, we call this **phagocytosis** (Figure 1.10). Phagocytosis is very important in defence against infection and indeed, in clearing up any kind of damage or cell death which has taken place in the body. Phagocytes have a biochemical arsenal available that can kill bacteria, fungi or parasites that they have phagocytosed.

Neutrophils

There are two major types of phagocyte, mononuclear phagocytes and neutrophils. The mononuclear phagocytes are long-lived cells that are distributed throughout the body, while the neutrophils are the kamikaze cells of the immune system. Neutrophils are produced in huge numbers in the bone marrow, migrate from there to the tissues, phagocytose material and die, all within about 48 hours.

Neutrophils belong to a group of cells called 'polymorphonuclear leukocytes' (Figure 1.6) or **polymorphs** for short, so called because the nucleus has several lobes. There are three types of cell in this category that are distinguished according to the way they stain with different dyes. The three types are:

- **neutrophils** that stain with neutral dyes and are mostly involved in antibacterial defence.
- **eosinophils** that stain with acidic dyes such as eosin, and which are important in defence against parasitic worms.
- **basophils** that are important in the control and development of inflammation.

Of these three cell types, only the neutrophils are phagocytes and they are by far the most abundant. Nearly 70% of the leukocytes in the blood are neutrophils. Unlike most other cells of the immune system that can traffic between the lymphoid tissues and the other tissues, neutrophils make a one way trip from the bone marrow to the different tissues of the body. However, they tend to accumulate particularly at sites of bacterial infection. For example, the pus that is produced in an infected wound contains huge numbers of neutrophils, which are attracted to the area by products released by the bacteria, and by cytokines released by cells of the immune system in their battle to control the spread of infection. This process is called **chemotaxis** and we shall look at it in detail later, when we also consider how phagocytes destroy microorganisms.

Mononuclear phagocytes

Let us turn now to the **mononuclear phagocytes** (Figure 1.6). There are representatives of this lineage in every tissue of the body. Some of them are fixed cells and some are mobile within the tissues. For example, in the liver there are a group of fixed phagocytic cells (Kupffer cells) which take up any material in the blood that passes through the liver. Every tissue needs a permanent population of phagocytes to clear up debris, and these fixed phagocytes may become activated for defence if the organ becomes infected. In addition, mobile phagocytes are important in patrolling through tissues. The precursor of this lineage is the **monocyte**, a cell type that constitutes about 5% of the leukocytes in the blood. Monocytes can migrate out of the blood vessels into tissues where they differentiate into **macrophages**, for example the lung macrophages. Like the neutrophils, macrophages have a powerful but distinct range of mechanisms for killing bacteria and parasites. But unlike the neutrophils they are long-lived and have several important additional functions, one of which is antigen presentation. Mononuclear phagocytes are able to take antigens that they have internalized, process them and present them to T_H1 cells.

- ☐ Recall how T cells recognize antigen, using their T cell receptor. What does the receptor recognize? From this, you should be able to predict two of the events that occur as a macrophage processes antigen for recognition by T_H1 cells.
- ☒ T cell receptors recognize antigen fragments bound to MHC molecules. So the antigen processing involves firstly the fragmentation of the antigen, and secondly the combination of antigen fragments with MHC molecules.

1.2.3 Antigen-presenting cells

The description of macrophages above reintroduced the idea of antigen presentation. Macrophages are just one of a group of cells which can perform this function. For example, B cells can act as antigen-presenting cells for T_H2 cells. So the definition of an **antigen-presenting cell** is a functional one; it is not the description of any particular cell lineage. Effectively, it means any cell that can

present antigen to a T helper cell. The term is so often used in immunology that it is very frequently abbreviated to APC.

We can see the basis of two types of cellular interaction here (Figure 1.11):

- 1 Macrophages present antigen to Th1 cells, and Th1 cells help macrophages to destroy the pathogens they have phagocytosed.
- 2 B cells present antigen to Th2 cells, and Th2 cells help the B cells to make antibody.

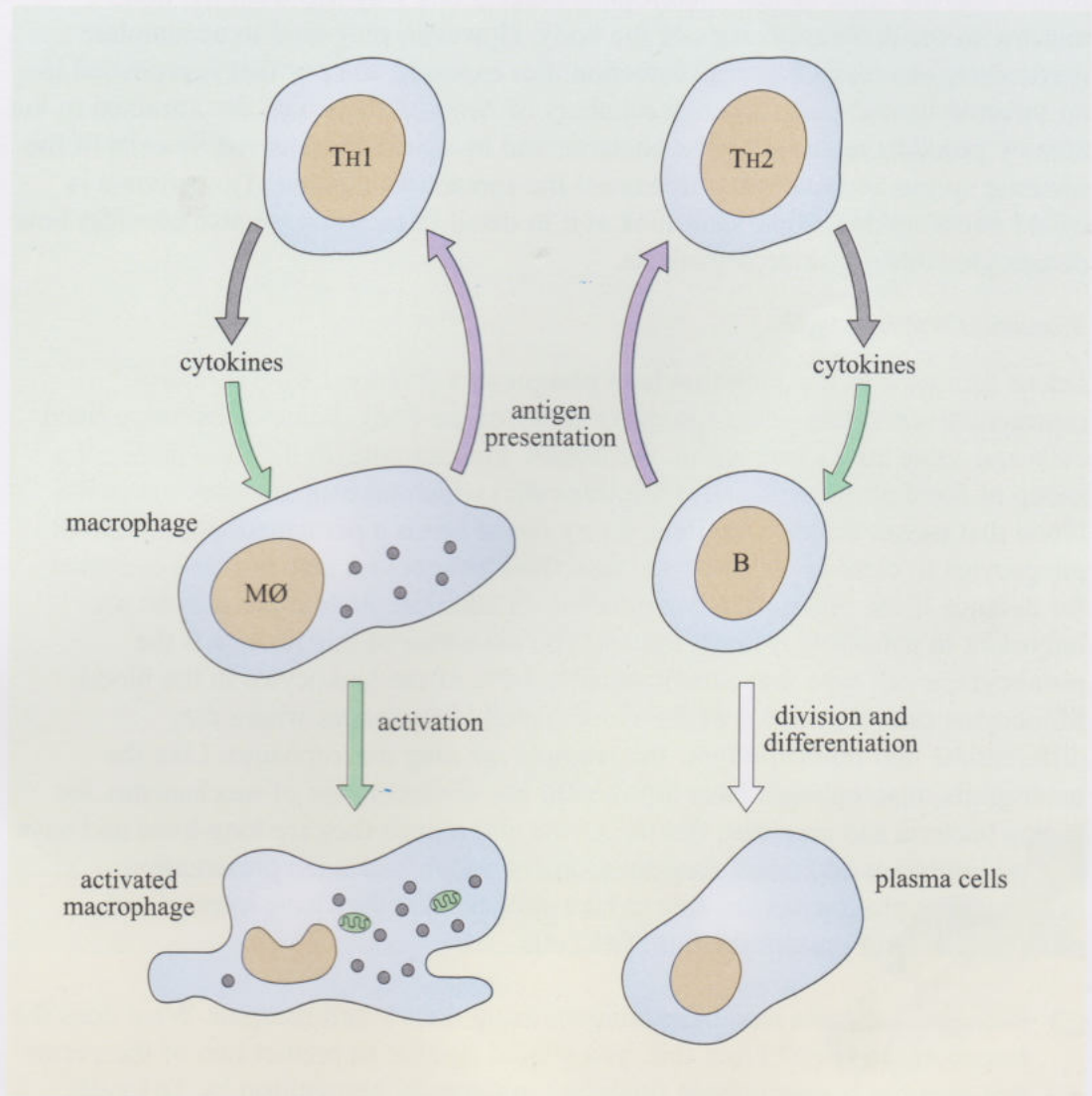


FIGURE 1.11 Macrophages present antigen to Th1 cells. They respond to cytokines released by Th1 cells by enhanced microbicidal activity. B cells present antigen to Th2 cells and respond to cytokines released by these cells by dividing and differentiating into antibody-producing plasma cells. The diagram shows two basic patterns of reactivity seen in immune responses. In practice, most immune responses display both types of response although they are often polarized to a Th1 or a Th2 response. Moreover, there are important interactions between the two sets of cells and the responses they mediate.

Dendritic cells

In addition to macrophages and B cells, there is another group of cells called **dendritic cells** (Figure 1.6) that are very effective in presenting antigen to T cells. In the context of immunology, dendritic cells are a specific group of leukocytes, derived from stem cells in the bone marrow. (The reason for pointing this out is that the term 'dendritic' describes the shape of a cell that has numerous filamentous processes. Since many cells in the nervous system are dendritic in shape, neuroscientists often use this term descriptively of nerve cells, but this is not the same as a leukocytic dendritic cell). Dendritic cells are very effective at presenting antigens to T cells, including naïve T cells, which have not previously been activated by antigen, something that macrophages and B cells do less well. Typically, dendritic cells are found in lymphoid tissues, where they are surrounded by T cells. Cells that belong to this lineage are also found in the blood, in very small numbers, and in the lymph.

Dendritic cells can also occur in non-lymphoid tissue. For example, dendritic cells are found in the skin where they are called **Langerhans cells**. If an antigen contacts the skin, they can take it up, migrate out of the skin, and pass through the lymphatic channels to the local lymph nodes carrying the antigen. They then process the antigen and present it to T cells. When you look at the animation of cell traffic on CD1, you will notice that lymph nodes have different regions. T cells tend to localize in one area and B cells in another. Not surprisingly, the dendritic cells localize in the T cell areas in order to present their antigen to the T cells there.

Follicular dendritic cells

We must finally introduce one more type of antigen-presenting cell – the **follicular dendritic cell (FDC)**. These cells are different from dendritic cells and are permanently located in the B cell areas of lymphoid tissues, where they present antigen to B cells. They are important in the process of antibody production, and we shall return to them later.

1.2.4 Auxiliary cells

Many cells of the body take part in immune reactions, by producing or responding to cytokines or by interacting with lymphocytes. There is, however, a smaller group of cells that contributes to the control of inflammation and these cells are often called auxiliary cells. Of these, the **mast cell** is particularly important. They lie close to the blood vessels in virtually all tissues of the body and contain numerous granules, full of mediators that promote inflammation, such as histamine that causes increased blood flow to an area. When they are activated, **mast cells release the contents of their granules**, thereby inducing inflammation (Figure 1.12). They can also synthesise mediators (signalling molecules) which cause leukocytes to be attracted to a site of inflammation. This process is called *chemotaxis* and the mediators are *chemoattractants*. Mast cells are related to basophils that also contain inflammatory mediators. Basophils can be triggered in a similar way to the mast cells, although basophils are generally confined to the blood.

Blood platelets also act as auxiliary cells, although these are not strictly cells, since they do not contain a nucleus. Platelets are produced by the fragmentation of a large cell in the bone marrow called a megakaryocyte, and they then circulate in the blood. Their principle role is in promoting blood clotting, but they also contain a

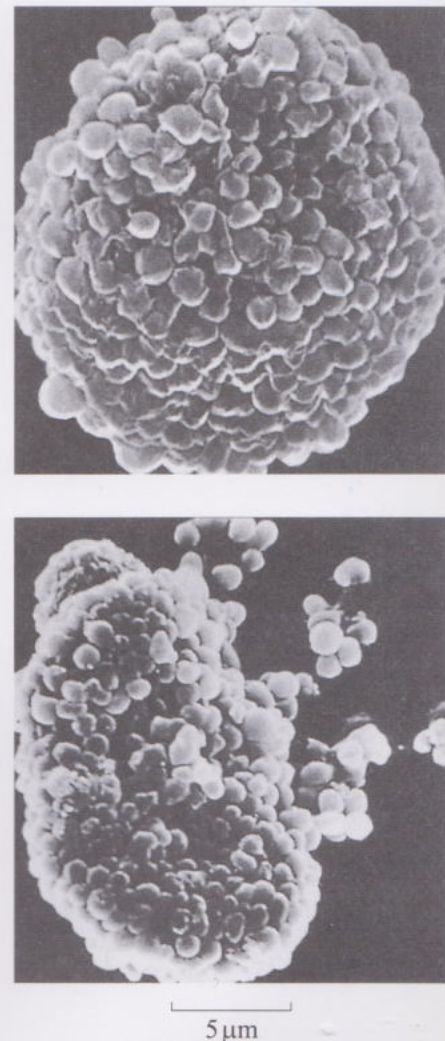


FIGURE 1.12 The scanning electron micrograph shows a mast cell (top). These cells are stuffed with granules containing mediators of inflammation, visible here as the plasma membrane has shrunk onto them. If the mast cell is activated, the granules and their contents are released (bottom).

number of inflammatory mediators and chemoattractants that are released as the platelets aggregate during blood clotting.

1.3 Cell traffic (CD1)



You have now encountered the main components of the immune system, including the different populations of lymphocytes and phagocytes, and the organs of the immune system. We have raised the idea of how cells migrate between lymphoid tissues as they patrol the body. This aspect is illustrated in CD1 in the animation entitled *Cell traffic* which is accessed via the Navihedron, using the symbol of a person.

You should work through this section paying particular attention to the types of cells, their locations and functions, and the ways in which they move between lymphoid tissues. Use the expansions (symbol 'i') to provide extra detail on the different cell types. Make sure that you see the video of 'chemotaxis and phagocytosis'.

Once you have worked through the section, try the self-assessment questions (accessed via the ✓ symbol). You should be able to answer most of the questions on cell function and traffic (1, 2, 3, 4, 7, 9, 10, 11, 12, 13, 14, 17, 18). If you need extra information use the 'i' button which will take you directly to the relevant pages of background information on the expansion screens. Detailed questions on the structures of the lymphoid organs are beyond the scope of this course.

You should take 60–90 minutes to work through this animation and the associated questions, more if you look up many of the expansion screens.

Summary of Chapter 1

- 1 The immune system has evolved different ways of protecting the host against different pathogens. The immune system appears complex, because it has to respond against a wide variety of pathogens and defence systems have evolved which are appropriate for each pathogen. The innate immune defences act in concert with adaptive immune responses mediated by lymphocytes.
- 2 The immune system can recognise both intracellular and extracellular pathogens.
- 3 The route of infection is important in determining the type of response that develops.
- 4 Leukocytes are distributed in lymphoid organs and tissues, which are strategically placed to protect different areas of the body.
- 5 Recognition of pathogens and their antigens is carried out by lymphocytes, which fall into two main subsets: B cells use surface antibody to recognise intact antigens in solution and on the surface of infected cells. Eventually they differentiate into plasma cells that produce a secreted form of the antibody. T cells use their T cell receptor to recognise processed antigen fragments presented by MHC molecules on the surface of other cells of the body.

6 T cells fall into three principle groups:

- TH1 cells release cytokines that activate phagocytes.
- TH2 cells help B cells to make antibody.
- Tc cells can kill infected cells of the body.

Large granular lymphocytes are also involved in recognising infected cells.

7 Phagocytes including neutrophils and macrophages internalize and destroy material that they have phagocytosed.

8 Antigen-presenting cells (APCs) are a functionally defined group that includes B cells, macrophages and dendritic cells. They present antigens to T helper cells.

Leukocytes migrate through the body in the blood and lymphatics, moving between lymphoid tissues and other tissues of the body. The route of migration depends on the type of cell.

Learning outcomes for Chapter 1

- 1.1 Define and use, or recognise definitions and applications of, each of the terms printed in **bold** in the text.
- 1.2 Give a basic description of the organs, tissues and cell types that comprise the immune system and how these are organised to combat pathogens entering the body via different infection routes.
- 1.3 Describe how the immune response develops when the immune system encounters antigen.
- 1.4 Explain the requirement for the differentiation of immune system cell lineages and detail the development and functions of the lymphocyte classes.
- 1.5 Describe how the different immune system cell types migrate between lymphoid tissues as they patrol the body.

2 IMMUNE DEFENCES AGAINST INFECTION

This long chapter covers the full range of immune defences that the body uses to recognize and react against different pathogens. It is the main section of this book. However, it is interspersed with numerous visits to the *Immunology Interactive* CDs. These act as the natural breaks in the chapter and will also allow you to consolidate your understanding at each stage.

2.1 Antibodies

2.1.1 Antibody classes

As you saw in the last chapter, B cells use a cell surface antibody molecule to recognize antigens, and if they become activated they will go on to produce a secreted form of that antibody. Secreted antibodies are essential components of immune defence, performing a variety of functions, but the primary function is always to recognize and bind to antigen. In fact there are several different types of antibody, described as antibody classes. Each class of antibody has a distinctive structure and function. Antibodies are also called immunoglobulins (abbreviated to Ig) and the five antibody classes are called IgA, IgD, IgM, IgG and IgE. Briefly, the function of the different classes is as follows:

- IgA is the main antibody in mucous secretions. It protects against infections of the gut, respiratory tree and the genitourinary tract.
- IgG is the main antibody in blood and tissue fluids. It can directly neutralize many toxic molecules, prevent bacteria and viruses from attaching to cells, and it acts as an adaptor molecule for phagocytes allowing them to recognize and internalize pathogens (one part of the molecule binds to the pathogen and another part binds to the phagocyte). IgG has many other functions in immunity, including protection of the foetus and newborn, since it is the only antibody class which crosses the placenta.
- IgM is the first antibody to be produced in an immune response.
- IgD is involved in activation of B cells, but does not have a major direct role in defence against pathogens.
- IgE is involved in the development and control of inflammation.

We shall return to the functions of antibodies in detail later, but first we should look at their structure and how it is related to function.

2.1.2 Antibody structure

All antibodies have the basic 4-chain structure which we encountered earlier (Figure 1.7). We will take IgG as a prototype. It is the heavy chains of the molecule that determine the class of the antibody. So IgG molecules have one type of heavy chain (γ) while IgA molecules have another (α) and so on. The light chains of IgG are folded into two globular domains and the heavy chains into 4 domains. If one

looks at the amino acid sequence of antibodies that are derived from different clones of B cells, it is found that the domains that lie at the distal tips of the molecules are highly variable and are therefore called V-domains. It is these V-domains, one from the heavy chain and one from the light chain, which form the antigen-binding sites (Figure 2.1).

Closer examination of the structure shows that the variability within the V-domains is mostly located in loops of polypeptides found at the antigen-binding site. There are three of these loops in the heavy chain V-domain and three in the light chain V-domain (Figure 2.2). So the binding site of an antibody is formed by six highly variable loops, which are hence called hypervariable regions. Notice however, that since any single antibody has identical heavy and light chains, it will have two identical antigen-binding sites. It is the amino acid sequence in these hypervariable loops that determines the structure of the binding site and hence what epitope an antibody will recognize.

FIGURE 2.1

The four polypeptide chains comprising antibody molecules are folded into domains. The variable domains of the heavy chain and light chain (V_H and V_L) form the antigen-binding sites. The remaining domains of heavy and light chains are relatively constant (C domains). The hinge region confers segmental flexibility on the molecule. IgG antibodies can be split into two fragments by enzymic digestion. The antigen-binding fragment of the antibody, known as the Fab region, consists of two domains from the heavy chain and two from the light chain, while the Fc region can bind to receptors on different populations of leukocytes.

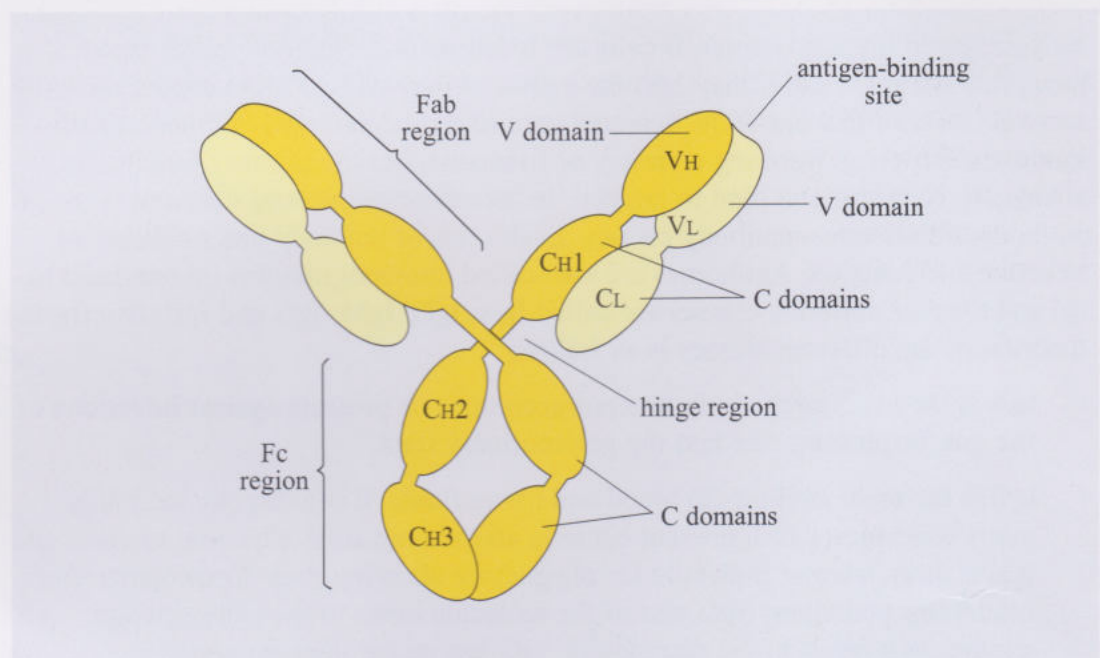
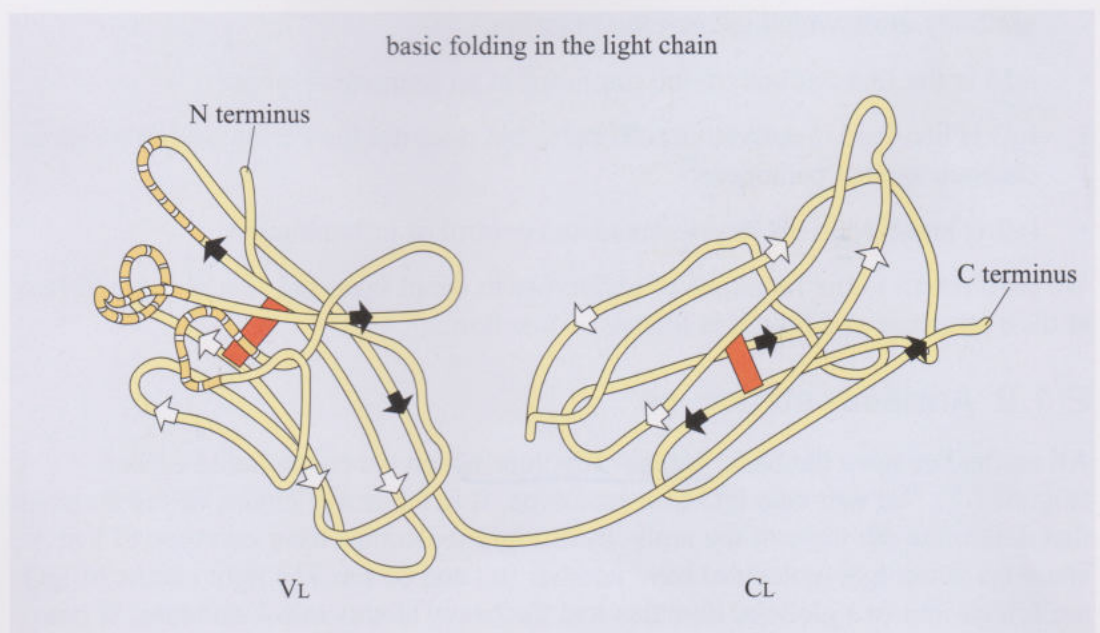


FIGURE 2.2

The folding pattern of the polypeptide chain of the variable and constant domains of a light chain is shown. Each domain is stabilized by disulphide bonds shown in red. The folding of the variable domain causes three hypervariable loops (hatched) to become clustered at the tip of the molecule and these, together with three hypervariable loops from the heavy chain, produce the antigen-binding site and determine the antigen specificity of the antibody. (The arrow heads show folded areas of β -pleated sheet structure.)



The remaining domains of the antibody are much less variable, and so are called constant domains or C-domains. However even the C-domains vary to a limited extent, because, as we noted above, the different antibody classes have different heavy chains. Also, you know that each of these antibodies can be produced as a cell surface form that acts as a receptor on the B cell, or as a secreted form. These two types vary in the part of the molecule that attaches to the cell surface. Also of interest is the hinge region, which allows the molecule flexibility. This means that the two antigen-binding sites are not fixed in relation to each other, but can independently attach to two identical epitopes, such as might occur on the surface of a virus or bacterium.

When you next have the opportunity, look at the 3-dimensional model of an IgG molecule on the 'IgG structure (3D model)' expansion screen that can be accessed from the A-Z Index of screens (via the (i) button) on *Immunology Interactive* CD1. This will give you an idea of the overall shape of the molecule, and its underlying symmetry. You should be able to identify the heavy and light chains, the domains, the hinge region and the antigen combining sites.



We have taken IgG as the prototype, and noted that other classes of antibodies have different heavy chains. Sometimes the heavy chains can have five domains instead of four (IgM and IgE), but a more striking difference is that IgA and IgM are usually produced as polymeric forms of the basic 4-chain structure (Figure 2.3). IgA is usually dimeric and thus has four antigen combining sites, while IgM is pentameric and has ten.

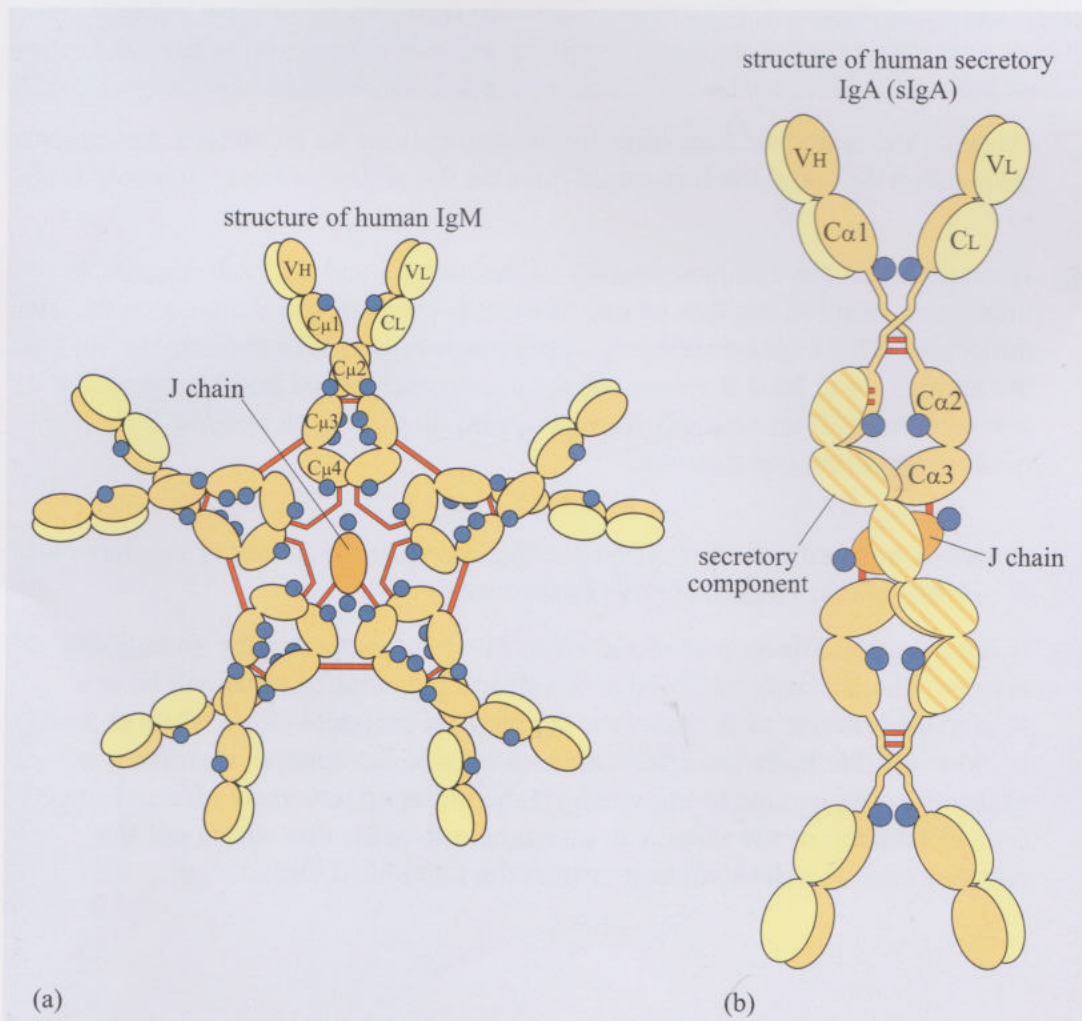


FIGURE 2.3

IgM consists of a pentamer of the basic 4-chain immunoglobulin structure and IgA is a dimer. Each is joined by an extra chain called the joining or J-chain. Notice that there are five domains in the heavy chains of IgM, while IgA, like IgG has four. When IgA is secreted an additional chain called the secretory component becomes bound to the IgA dimer. The positions of carbohydrate units are indicated as blue discs. Inter-chain disulphide bonds are shown in red.

Although the diversity of antibodies may seem complex, the principle underlying antibody structure is very simple: *one part of the molecule (V domains) binds specifically to epitopes on an antigen; other parts of the molecule interact with cells or other immune defences.* In other words, antibodies act as adapters.

2.1.3 How antibodies bind to antigens

When an antibody binds to an epitope the two molecules are seen to have complementary shapes. The shape of the epitope is determined by the molecular groups present on the surface of the antigen, while the shape of the antibody's binding site is determined by the amino acid residues in the hypervariable loops of the heavy and light chains. In addition to having complementary shapes, the antibody forms many weak non-covalent bonds (e.g. hydrogen bonds) with the epitope. The bonds that are formed depend on the molecular groups on the antigen and antibody. Although each individual non-covalent bond is weak, the combination of bonds produces a strong overall bond.



When you have a suitable opportunity, look at the molecular model of the protein lysozyme binding to a combining site on a specific antibody. In this case the lysozyme is acting as the antigen. The model can be accessed from the A-Z Index on CD1 under 'Fab/Lysozyme complex (3D model)'. This model shows just one part of the antibody, a light chain and part of the heavy chain. See also the still pictures of this model on the 'Antigen-binding site' expansion screen. The antigen and antibody interact with each other over an extended area that involves 16 amino acid residues on the antigen, and 17 on the antibody, some from the light chain and some from the heavy chain. Most of the contact residues in the binding site come from the hypervariable loops on the antibody.

- ☐ If one of the amino acid residues forming an epitope on an antigen was mutated what effect do you think this would have on the ability of the antibody to bind to it?
- ☒ It would affect the complementarity of the antigen and antibody shapes. It might also result in the loss of one of the non-covalent bonds between the two molecules. This might completely destroy the ability of the antibody to bind to the antigen, or at least it would reduce the strength of the bond between the two molecules. If an antibody loses its ability to bind to an antigen, it is ineffective in immune defence.
- ☐ If a virus mutated one of its surface antigens, what effect would this have on the ability of that virus to survive in the host?
- ☒ If the mutation affects part of the virus which is recognized by an antibody (an epitope), the antibody will bind less well and the mutated virus will be at a selective advantage as it can evade the immune response (for a while at least). If, however, the mutation affects an area outside the epitope, then there is probably no advantage to the virus. (If the mutation adversely affected some critical function of the virus, e.g. attachment to cells, this would put the mutated virus at a disadvantage against the unmutated virus.)

2.1.4 Antibody affinity, avidity and cross-reactivity

The discussion above leads on to the ideas of **antibody affinity** and **avidity** (Figure 2.4). These terms have precise meanings and can be measured experimentally, but put simply, an antibody which makes a strong bond with its antigen – many non-covalent bonds, and a good complementary shape – is said to have a high affinity. Generally speaking, high affinity antibodies are better at combating infection than low affinity antibodies. Also, it is generally found that IgA, IgG or IgE have higher affinity than IgM antibodies. However, we now introduce the idea of avidity. This concept takes into account not just affinity (the strength of a single antigen/antibody bond) but also the fact that antibodies have several combining sites. Thus IgM can have a high avidity for an antigen, because it has ten combining sites, even though the individual binding sites are of low affinity. This is called the bonus effect of multi-valency. So IgM antibodies can be very useful in circumstances where a pathogen has a cluster of identical epitopes on its surface. For example, the surface of a bacterium may have millions of identical carbohydrate epitopes and an IgM molecule can make several bonds with it. This is where the segmental flexibility of the hinge region is important, allowing the IgM to bend so that several epitopes can be bound simultaneously.

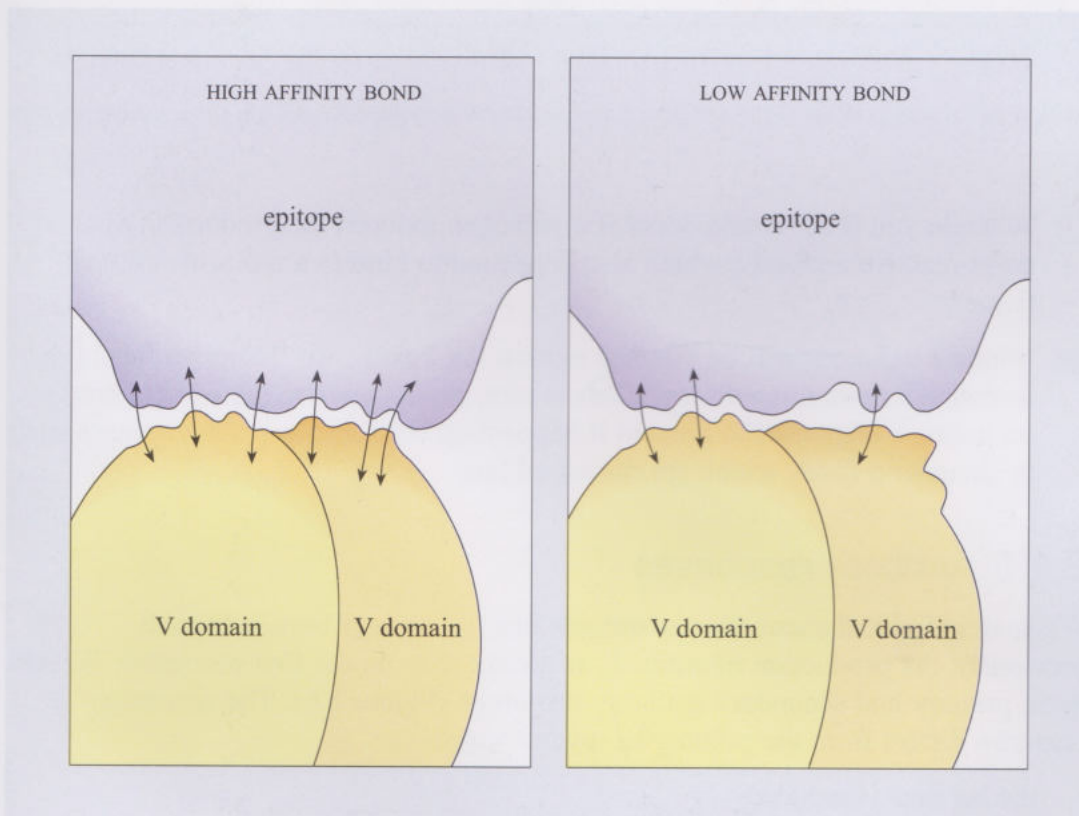


FIGURE 2.4

Antibodies form numerous non-covalent bonds with the epitope on the antigen that they recognize. Although individually weak, the combined bond is strong (high affinity). If however the fit is less good, fewer and weaker non-covalent bonds form and the bond is of low affinity.

One more idea needs to be introduced here – **cross-reactivity** (Figure 2.5). Up to this point we have said that each antibody binds to one antigen and only one antigen, and in most cases this is effectively true. However, you now know that an antibody recognizes a molecular shape. It is just possible that the same molecular shape (epitope) could appear on two different antigens. In this case the antibody would not be specific for the one antigen. It would bind to both, and we describe such antibodies as cross-reactive. This is not generally a problem: indeed it could be quite useful to have antibodies which recognize several different strains of the same pathogen. However, there is one circumstance where cross-reactivity can lead to major problems in immune defence.

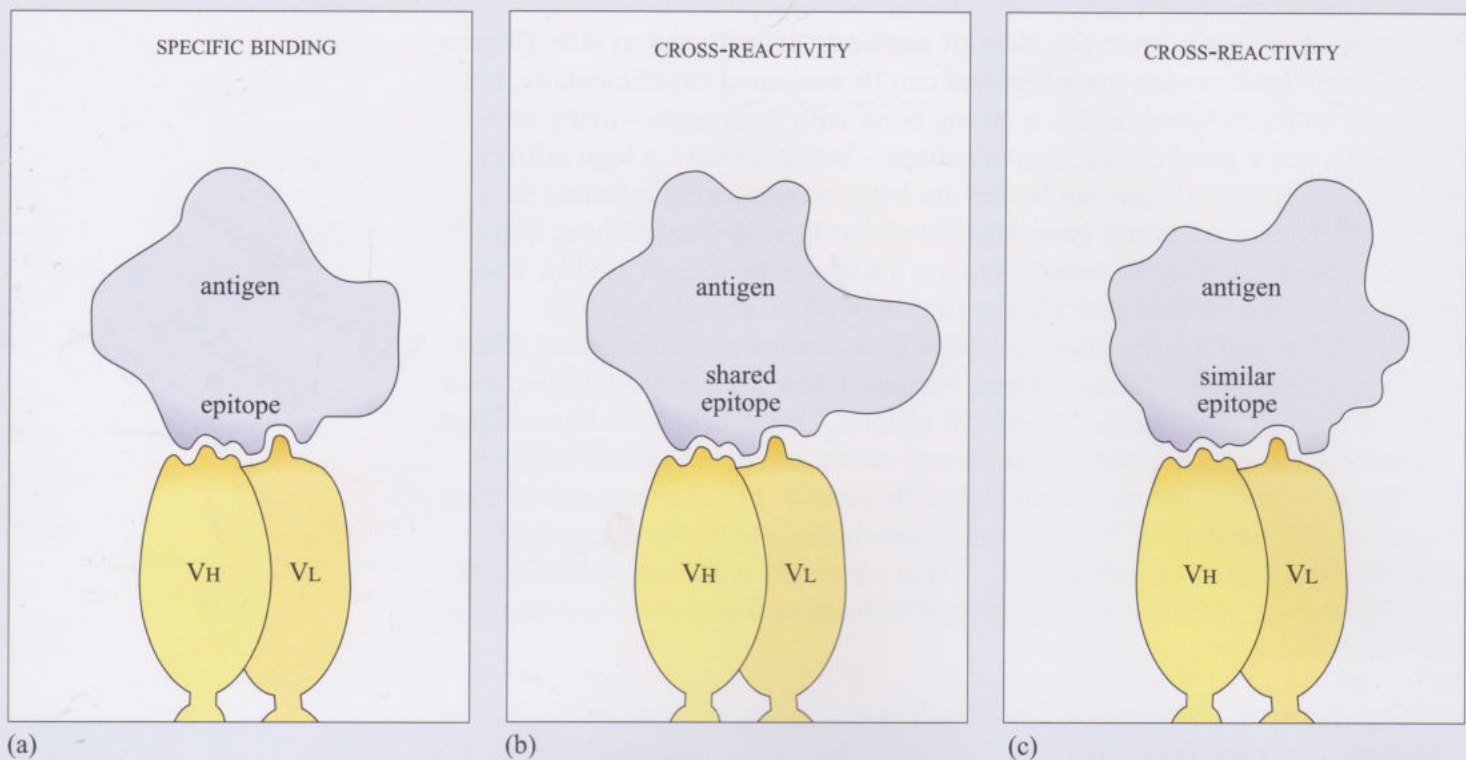


FIGURE 2.5 Antibodies may cross-react with other antigens either if the antigens have an identical epitope (a and b) or if a similar epitope occurs on another antigen (c).

- ☐ What do you think would occur if a pathogen induced the production of a cross-reactive antibody, which also happened to bind to a molecule on host tissue?
- ☒ Immune defences will be directed against the host's own tissue, producing a so-called autoimmune disease. This is rare, since there are numerous controls on immune responses to prevent it happening, but it can occur in diseases such as rheumatic fever, which are discussed later.

2.1.5 Antibody responses

When an individual encounters an antigen that has already been contacted previously, the production of antibody is greater than on the first encounter. We call these primary and secondary antibody responses (Figure 2.6). The secondary response differs from the primary in several ways:

- the lag time is reduced;
- the overall level of the antibodies is much higher;
- the antibodies persist for longer;
- there is a switch from IgM production to IgG;
- the affinity of the antibodies is generally higher.

All of these effects mean that the secondary response is more efficient than the primary, and this is the basis of vaccination. Immunization aims to induce a primary antibody response using a harmless agent (e.g. an attenuated strain of a microorganism which is not pathogenic), so that if the real pathogen is

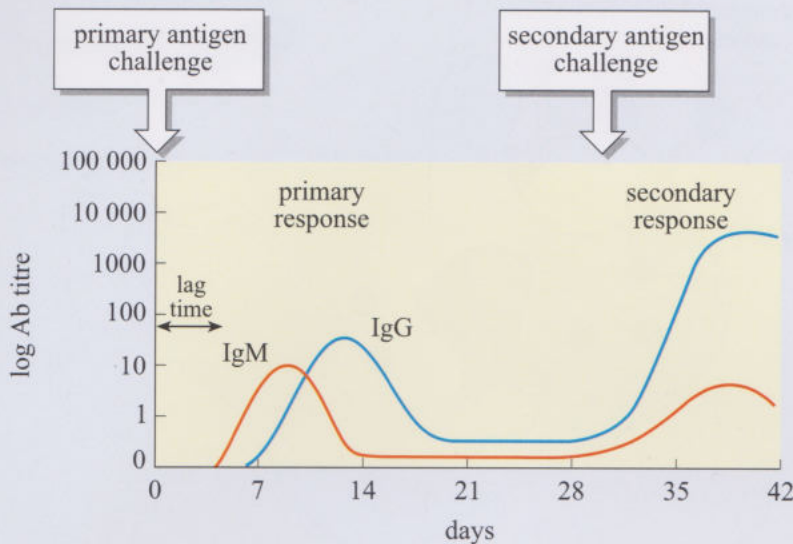


FIGURE 2.6
Characteristics of primary and secondary antibody responses.

encountered, a strong secondary immune response will develop immediately which can stop it before disease develops. (Note that the terms immunization and vaccination are used interchangeably, and that this area will be discussed in detail in Book 7 *Treatment and control*.)

2.1.6 Functions of antibodies

Some functions of antibodies depend solely on their ability to bind to antigen. For example, if an antibody binds to a receptor on the surface of a virus, then it may prevent the virus from attaching to a host cell. More often however, it is necessary to recruit other immune defences. We have already noted that different antibody classes have different functions, related to the type of heavy chain they possess, and this is dependent on the Fc region of the molecule (see Figure 2.1). All cells of the immune system have receptors for the Fc region of antibodies (**Fc receptors**), but there are many types of Fc receptor, each one being specific for a particular class of antibody. Each cell type has its own set of Fc receptors, which means that it can bind to a particular set of immunoglobulin classes which mediate a range of functions. This will become clearer when we describe some examples later in this chapter.

Opsonization

Opsonization is the process by which a molecule such as an antibody helps phagocytes to recognize a pathogen, and facilitates phagocytosis. Mononuclear phagocytes and neutrophils have receptors on their surface which bind the Fc region of IgG (Fc γ receptors). Antibody bound to the Fc receptor and to the pathogen (via the antigen-binding site) acts as a cross-bridge between the phagocyte and pathogen and facilitates phagocytosis (Figure 2.7). Immune complexes (soluble antigens bound to antibody) can also be taken up by this mechanism.

There are three main receptors for IgG (Fc γ RI, Fc γ RII and Fc γ RIII) distributed on different leukocyte populations. All mononuclear phagocytes express Fc γ RI, and Fc γ RIII – these are the receptors that the macrophage uses for binding to

The names of the receptors for antibodies are pronounced as follows:
 Fc γ RIII (eff-see-gamma-are-three)
 Fc ϵ RI (eff-see-epsilon-are-one), etc.

FIGURE 2.7

Antibodies can opsonize antigens for uptake by phagocytes. This includes immune complexes of antigen and antibody and pathogens such as bacteria. The bound antibody binds to Fc receptors on the surface of the phagocyte.

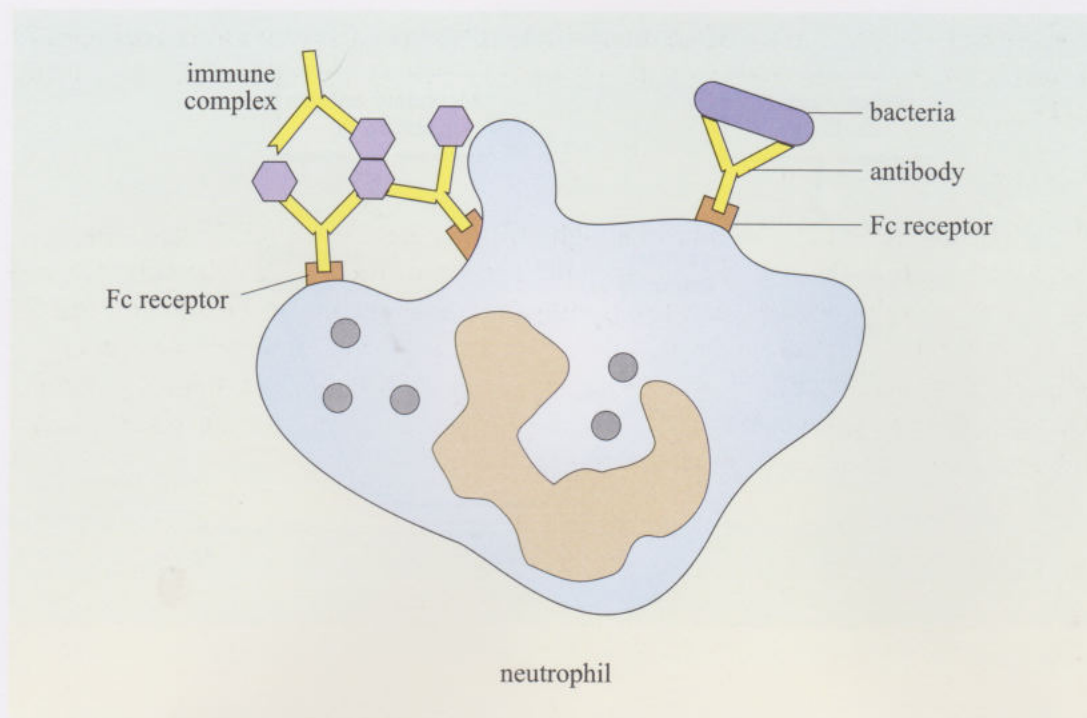


FIGURE 2.8

Different Fc receptors bind to different types of antibodies. Receptors for IgG are shown on the left and receptors for IgE on the right. The three IgG receptors and the first IgE receptor Fc ϵ RI, are folded into domains which are structurally similar to the domains in antibodies, while the other IgE receptor Fc ϵ RII belongs to a different family of molecules. The receptors are distributed on different cell types as indicated.

IgG receptors			IgE receptors	
				
Fc γ RI	Fc γ RII	Fc γ RIII	Fc ϵ RI	Fc ϵ RII
monocytes	monocytes neutrophils B cells	monocytes neutrophils NK cells	mast cells basophils	B cells T cells eosinophils monocytes

opsonized antigens. Fc γ RI (CD64) is a specific marker of mononuclear phagocytes, but Fc γ RIII is also present on most large granular lymphocytes, and they can use it to recognize antibody bound to infected cells. Fc γ RI and Fc γ RIII are present on neutrophils that also use the receptors for recognising opsonized antigens. In effect, when antibody binds to an antigen, it labels it for phagocytosis. Another receptor, Fc γ RII, is present on B cells but this has quite a different function. It allows a B cell to respond to the level of antibodies in solution and adjust its own production of antibody accordingly. The structures of some of the different Fc receptors are shown in Figure 2.8.

Receptors for other classes of antibody (IgA, IgM, IgE) can also allow leukocytes to recognize pathogens, for example IgE receptors (Fcε) on eosinophils allow them to engage helminths.

Control of inflammation

Basophils and mast cells both have a high affinity receptor for IgE (FcεRI) and most of the IgE in the body is bound to the surface of these cells. The cells are said to be sensitized by the IgE, because if antigen cross-links the surface-bound IgE it causes the cells to release their granules, which contain inflammatory mediators (Figure 2.9), and to synthesize new mediators. We shall consider inflammation and the role of mast cells and basophils later. At this stage, it is sufficient to know that IgE antibodies are involved in inflammation.

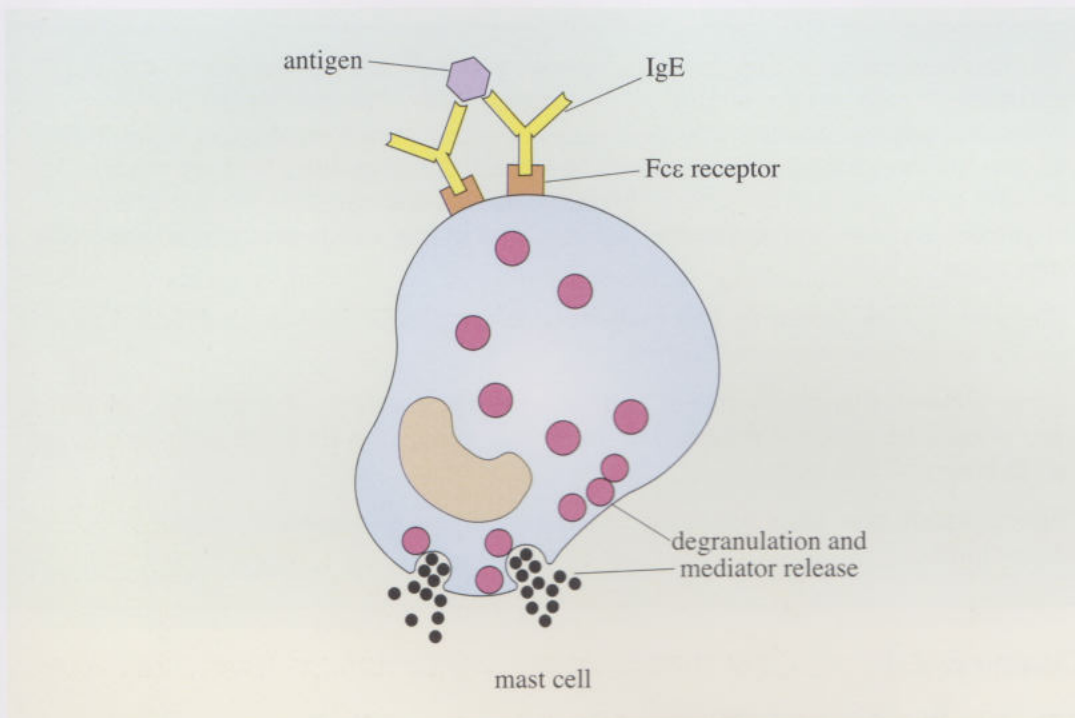


FIGURE 2.9

IgE binds to IgE receptors on the surface of mast cells, thus sensitising them to antigen. If antigen cross-links the IgE and thereby the receptors, the mast cell is triggered to degranulate, releasing the inflammatory mediators in its granules.

Activation of complement

One important component of immune defence is the **complement system**, a group of about 20 proteins, present in blood and tissue fluids, which interact with each other and with many cells of the body, carrying out a number of protective functions. The complement components are always present, although their synthesis may be increased during infection. We shall return to the complement system later, but we should note here that IgG and IgM antibodies are able to activate complement functions by binding to the first component of the system, C1. Although C1 is not an Fc receptor in the same sense as the others described here, it does recognize and bind to the Fc portion of antibodies and is therefore effectively a soluble receptor for antibodies.

In each case above you can see that the antibody acts as an adapter by cross-linking the antigen to a cell or another immune defence system component.

- ☐ In immune responses against helminths, the body often produces high levels of specific IgE. In responses against enteric bacteria, a strong IgA response occurs, and in responses to malaria, IgG is the major antibody. Can you see any reason (advantage) in producing different classes of antibodies, in different infections?
- ☒ The appropriate type of immune response depends on the pathogen. A response that is effective against one pathogen can be useless or even detrimental against another. Each of the antibody classes mediates a different type of immune defence. The body tends to synthesize the class of antibody that is most appropriate for dealing with each type of infection.

2.2 Influenza mini-lecture (CD2)



You are now going to look at the immune response against influenza. Although cytotoxic T cells are important in destroying virally-infected cells, the antibody response against the virus is also very important since antibodies which bind to the haemagglutinin can prevent the virus from attaching to cells, thus limiting the spread of infection. However, as you will see, the virus mutates regularly, so that the antibodies against one strain are ineffective against the new strain.

You should now listen to the mini-lecture entitled *Influenza* located on disc 2 (CD2) of *Immunology Interactive*.

After listening to the lecture, and with your knowledge of antibody functions, you should be able to answer the following questions (the answers are given at the end of the book).

Spend about one hour studying the mini-lecture and answering the questions.

Question 2.1

Why do most of us catch flu several times during our lives?

Question 2.2

Why do the antibodies against one strain of flu not protect us against another? Can you explain the molecular basis of this observation?

Question 2.3

Why do antibodies against the haemagglutinin limit the spread of infection whereas antibodies against the M-protein do not?

Question 2.4

Why do some parts of the haemagglutinin mutate at a very high rate, while other parts do not? Why does the M-protein not mutate at all?

Question 2.5

Why do major pandemic strains generally produce much more serious disease than the variant strains that arise each year?

Question 2.6

Although the annual vaccine developed against flu is usually very effective, providing up to 90% protection, some years it is much less effective or ineffective – why is this?

2.3 Complement and inflammation

The complement system is a group of proteins that have a variety of functions in controlling inflammation and in immune defence. They interact with each other in an enzyme cascade, where the product of one reaction acts as the enzyme which catalyses the next. This is conceptually similar to the blood clotting system – a small initial trigger can precipitate a fast-growing chain of reactions. Complement system molecules are produced constitutively by the liver, although they can also be produced locally at sites of inflammation by macrophages. The principal functions of the complement system are:

- attraction of phagocytes to a site of inflammation (chemotaxis);
- opsonization of pathogens for phagocytosis, i.e. acting as an adapter between pathogen and phagocyte;
- opsonization of immune complexes for uptake by phagocytes;
- activation of the phagocytes to destroy material they have taken up;
- triggering of mast cells to release their inflammatory mediators;
- lysis (osmotic rupture) of cells and some bacteria and viruses which have been recognized as foreign.

2.3.1 Pathways of complement activation

The complement system can be activated via three different pathways (Figure 2.10), all of which converge on the central reaction of the pathway, the activation of C3. When C3 is activated it is cleaved enzymatically into two fragments, the larger, C3b, is involved in opsonization and the smaller, C3a, can activate mast cells. The pathways all generate enzymes (C3 convertases) which cleave the C3, but the triggers for each pathway are different. The **classical pathway** is activated by immune complexes containing antibodies of the IgM or IgG classes. (The classical pathway is so-called because it was the first to be discovered.) The **lectin pathway** is activated by carbohydrate groups that are typically found on the surfaces of bacteria and some fungi. Activation of the **alternative pathway** is a bit more complex; this pathway is continuously being activated, but only at a very low level. Cells of the body have a group of molecules on their surfaces that limit the activation of complement. They are referred to as complement control proteins. Bacteria and fungi lack the control proteins, so the alternative pathway is rapidly activated in their presence – they are said to have ‘activator surfaces’. So the alternative and lectin pathways act as a recognition system for microorganisms, while the classical pathway is directed to attack whatever the antibodies recognize.

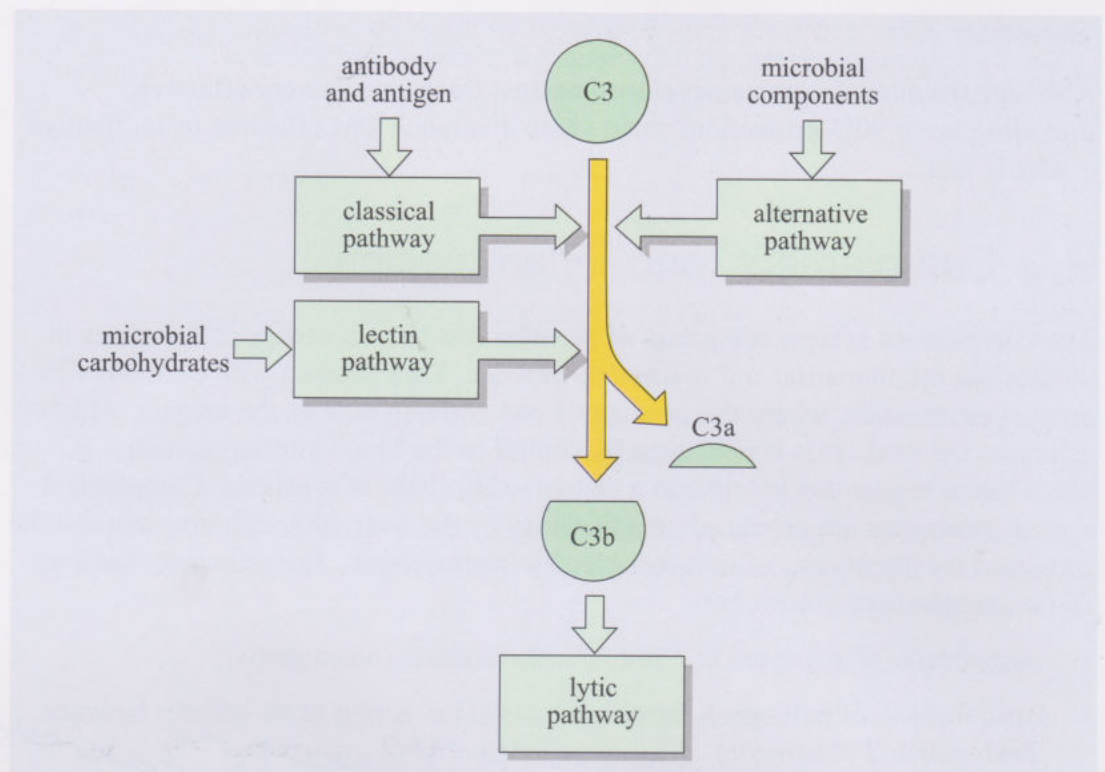


FIGURE 2.10 The arrangement of the different activation pathways of complement (classical, lectin and alternative pathways) is shown. Each pathway leads to the activation by cleavage of C3, into a large fragment C3b and a small fragment C3a. The activation of C3 may lead to the initiation of the lytic pathway.

When C3 is cleaved it exposes a very reactive bond on C3b, which can bind covalently to chemical groups present on proteins and carbohydrates. However, because the active bond is very transient, it means that C3b will only bind in the immediate vicinity of whatever caused the activation. This is usually an antibody combined with an antigen, or the surface of a microorganism (Figure 2.11).

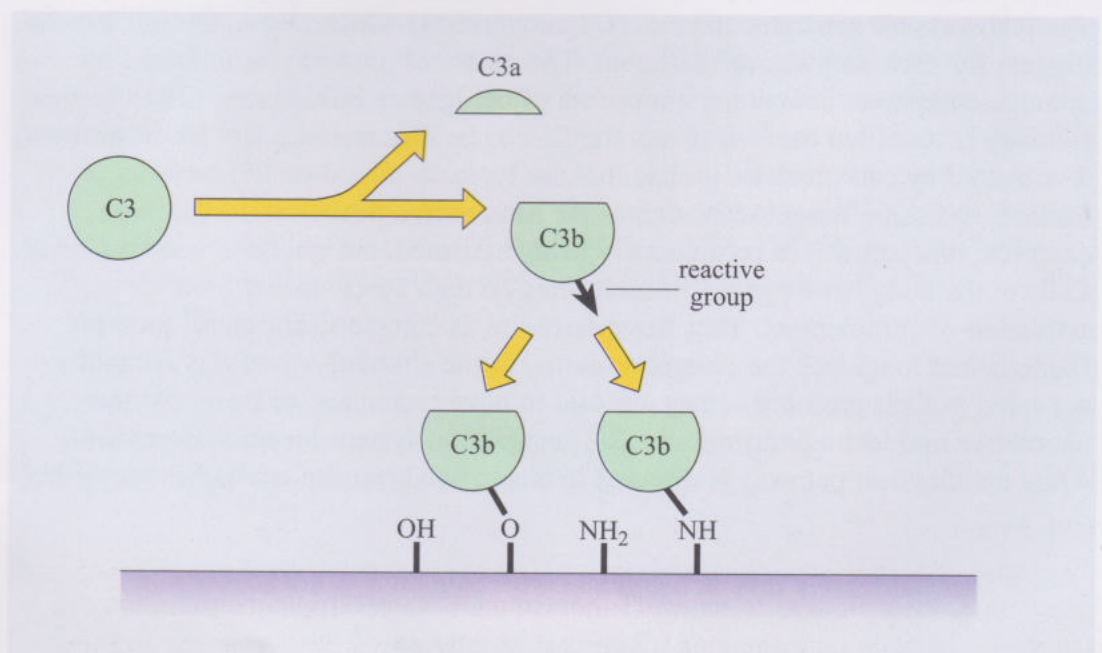


FIGURE 2.11 When C3 is split, a highly reactive bond is revealed on the larger fragment C3b. This can bind covalently to hydroxyl groups (-OH) or amine groups (-NH₂) present on nearby proteins or carbohydrates. This tags the molecule/pathogen, so that it can be recognized by cells that have receptors for C3b. If the reactive group does not combine quickly with a protein or carbohydrate, it decays and becomes inactive.

When antibodies bind to antigens they form **immune complexes**, these are multimolecular complexes containing a number of molecules of antigen bound to specific antibody. If the complexes activate complement with the formation of C3b, it too may become bound to the complex. One of the functions of complement is to keep immune complexes in solution (i.e. to prevent the formation of huge insoluble aggregations of antigen and antibody), so that they can be taken up by phagocytes.

If C3b has become bound on the surface of a pathogen, it can recruit additional complement proteins, belonging to the **lytic pathway**, which assemble a multimolecular complex called the membrane attack complex (MAC). On plasma membranes, this complex appears like doughnut-shaped channels through which the contents of the cell can leak. It also allows other components of the immune system access to the inside of a pathogen.

- ☐ What kinds of pathogens have a lipid bilayer exposed on their external surface, and might therefore be susceptible to damage by the membrane attack complex?
- ☒ Gram-negative bacteria have an outer membrane, and enveloped viruses have a plasma membrane derived from the infected cell. Note however that the inner plasma membrane of Gram-positive and Gram-negative bacteria is beneath the bacterial cell wall and is not normally exposed to this kind of attack. Eukaryotic pathogens, including worms and protoctists also have an external plasma membrane, so are susceptible to the lytic pathway of complement.

2.3.2 The functions of complement

After this brief introduction to the complement system, you should now work through the topic entitled *Complement* on CD1. The animation is accessed through the Navihedron icon of the molecule C1, which interacts with antibodies to activate the classical pathway. This molecule has six binding sites for antibody and is often said to resemble a bunch of tulips.

As you work through the animation you will get more information on the different molecules involved in each of the pathways. Although the details of the reactions are beyond the scope of the course, you may find that the processes are clearer if you can follow the reactions. A scheme of the reaction pathways is given on the expansion screen 'Complement reaction pathways' on CD1.

Within the animations, many different complement molecules are shown. To know what each of these molecules represents, use the label button. You may find it useful to visit the expansion screens on 'Chemotaxis', 'Mast cell degranulation' and the 'Antibacterial role of complement'.

After you have finished the topic look through the associated self-assessment questions. You should be able to answer Questions 1, 2, 3, 5, 7 and 8.

Spend about one hour on this activity.



Clearly, the deposition of C3b on the surface of pathogens targets them for phagocytosis by cells which have C3b receptors. Receptors for C3b are present on mononuclear phagocytes, neutrophils and NK cells. As these cells also have Fc receptors for IgG; this means that antibody and C3b can act together to opsonize

antigens and immune complexes. A different kind of C3b receptor is found on B cells and follicular dendritic cells and this receptor has an important role in the development of the antibody response. It allows these cells to accumulate antigen as immune complexes early in an immune reaction, something which is required for development of the secondary response, as you will see later.

- ☐ Occasionally, children are born who have a genetic deficiency of C3. What effect do you think this would have?
- ☒ These children are extremely susceptible to infection with Gram-positive bacteria, e.g. staphylococci and streptococci, because their macrophages do not phagocytose them efficiently.

The smaller fragments generated by complement activation, specifically C3a and C5a, have a central role in the development of inflammation, including the triggering of mast cells to release inflammatory mediators and in attracting phagocytes to a site of infection. This brings us to the subject of inflammation, its characteristics and what function it serves.

2.3.3 Inflammation

You are all familiar with the outward features of **inflammation**, including the pain, redness and swelling which often accompany an infection. These signs can be related to the underlying cellular processes that are taking place in the infected tissue. We can identify three principal components of inflammation (Figure 2.12):

- an increased blood supply to the area;
- an increase in the leakiness of the local capillary blood vessels;
- migration of leukocytes out of the blood vessel and into the tissue.

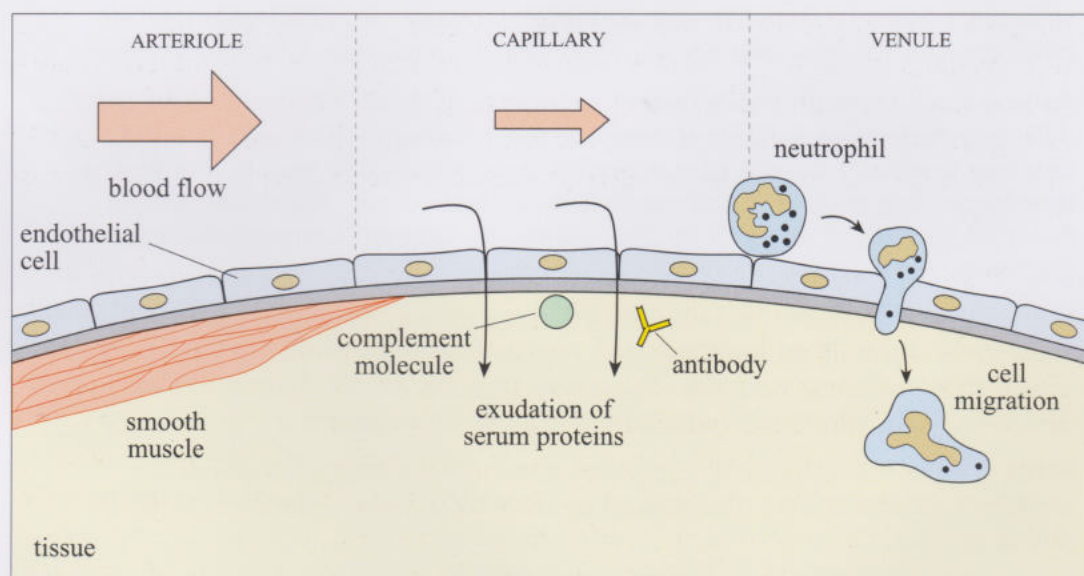


FIGURE 2.12 The three main features of inflammation are controlled at different parts of the vasculature. Smooth muscle in arterioles determines the diameter of the vessels and controls the blood flow through a site of inflammation. Exudation of serum molecules occurs in capillaries as endothelial cells retract in response to inflammatory mediators. Migration of leukocytes takes place in venules, partly because the shear force is lowest in the venules and partly because signalling molecules and adhesion molecules are expressed on the surface of the venular endothelium.

Each of these functions serves to bring cells and molecules of the immune system to the site of infection. The increase in blood supply means that there are more cells and serum molecules (including antibodies and complement molecules) arriving in the area. It is also responsible for the redness seen at a site of inflammation. The increase in leakiness of the blood vessels is more usually called increased capillary permeability. Normally most of the large serum molecules are retained within the serum, since the endothelial cells that line the internal wall of the blood vessels prevent large molecules from crossing. But at sites of inflammation, the endothelial cells retract and this allows the larger molecules to leak out in greater amounts. This is very desirable, since it is important to get antibodies and complement into the tissue to combat infection. Leakage of serum shows up as the swelling. The migration of leukocytes into the tissue is equally important, since they are needed at the site to phagocytose pathogens, or to attack infected cells in the tissue. So, the function of inflammation is to get appropriate immune defences to a site of infection.

The process of inflammation is very carefully choreographed by signalling molecules (cytokines) released in the tissue. The type of inflammation that develops does in fact depend on the type of infection and on the tissue involved. Remember that an appropriate immune defence depends on what infectious agent is involved. So, for example, an acute local infection of the skin with *staphylococcus* (a boil), will induce many neutrophils and macrophages to enter the tissue, whereas a chronic infection of the lung with *M. tuberculosis* will cause the inward migration of T cells and macrophages.

Cell migration

In order to understand how different types of leukocyte traffic develop in different conditions, we need to look at the underlying cellular and molecular processes that direct cell migration from blood vessels into the tissues. The key to this process is the interaction of the circulating leukocytes with signalling molecules and adhesion molecules that appear on the surface of the endothelial cells at sites of inflammation. Adhesion molecules include several families of molecules that allow cells to adhere to other cells or to components of the extracellular matrix (e.g. collagen). On the endothelium these include selectins, which slow circulating cells, and **cell adhesion molecules (CAMs)**, which mediate leukocyte migration across the endothelium.

Cell migration takes place across venules (small veins), partly because the shear force pushing cells through the circulation is lowest in venules, and partly because this is where the signalling molecules are located. The initial adhesion of leukocytes to the endothelium takes place in three phases (Figure 2.13). Initially the cells are slowed and roll along the endothelium. This gives them the chance to receive signals from molecules attached to the endothelial surface called **chemokines**, (a family of cytokines that are involved in chemotaxis and cell activation). Receptors for chemokines are distributed on different populations of leukocytes, and each population has a different profile of receptors. In addition, activation of a cell often changes the number and types of chemokine receptors that it expresses.

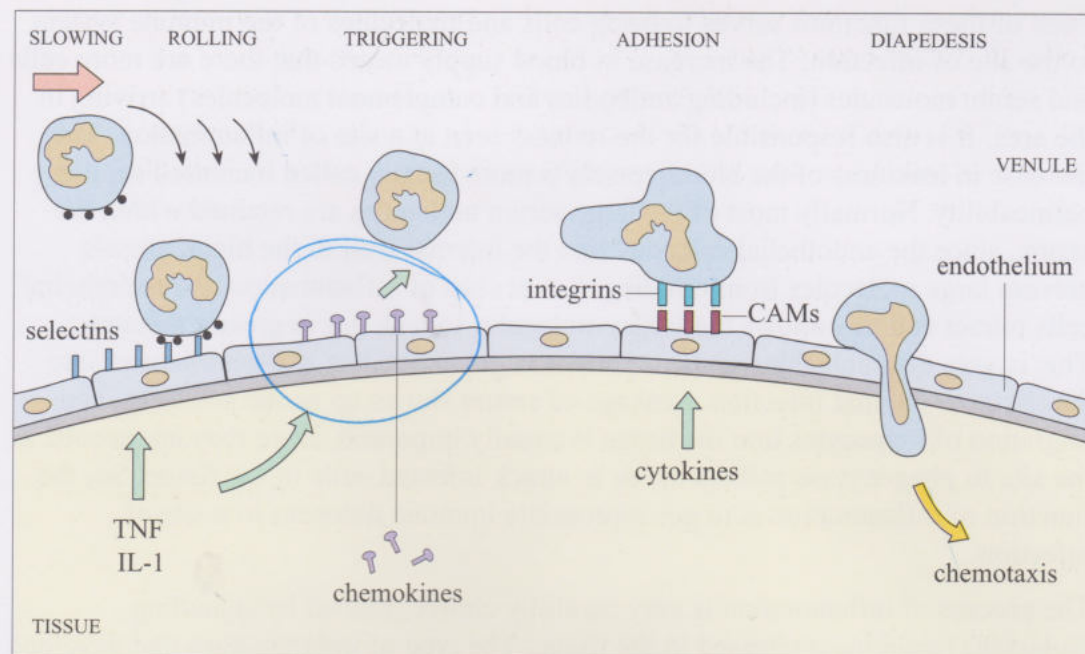


FIGURE 2.13 Circulating leukocytes are slowed by binding to selectins induced on the endothelium by inflammatory cytokines such as TNF and IL-1 produced in the tissue. The slowed leukocytes roll along the endothelium and can then respond to chemokines held on the surface of the endothelium. The chemokines may have come from cells in the tissue, or may be produced by the endothelium itself. Leukocytes which have been activated by chemokines or other chemotactic molecules reorganize adhesion molecules (integrins) on their surface, which bind to cell adhesion molecules (CAMs) induced on the endothelium by inflammatory cytokines. With a firm attachment to the endothelium the leukocyte can pull itself down through the endothelium, a process called diapedesis.

A leukocyte that has been activated by a chemokine will reorganize and activate molecules on its surface that belong to the family called **integrins**. (This is a group of adhesion molecules, which are expressed on leukocytes, and which can bind to different cell adhesion molecules on the endothelium; examples which are mentioned on the animation that follows are LFA-1 and VLA-4.) The integrins can now interact with cell adhesion molecules that have been induced on the endothelium by inflammatory cytokines. Once a leukocyte has attached to the endothelium, it can pull itself down through the endothelium and make its way into the tissue where it can participate in the immune reaction.

You can see this process on the expansion screen 'Migration of lymphocytes across endothelium in vitro' on CD1 when you come to this topic shortly.

The role of inflammatory cytokines

How does the tissue signal to the endothelium to promote leukocyte migration into an area of infection? At such sites, inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor- α (TNF α) are produced which activate the endothelium locally. The inflammatory cytokines induce both the selectins that slow the leukocytes and the cell adhesion molecules that bind to the leukocyte integrins (Figure 2.13).

Mononuclear phagocytes, which are present in most tissues, produce IL-1 and TNF α in response to infection or cell damage. Another important cytokine is interferon- γ (IFN γ) which is produced by activated TH1 cells. This means that a T cell that has entered the tissue and encountered its antigen is able to signal to the endothelium to display adhesion molecules that are required for the migration of more leukocytes.

Although the adhesion molecules are essential for migration, it is the chemokines that principally determine which cells will migrate where. There are a large number of different chemokines, each of which promotes the migration of a particular group of leukocytes. For example, monocyte chemotactic protein-1 (MCP-1) promotes the migration of monocytes. Chemokines can be synthesized by cells in the tissues, or by the endothelium itself in response to inflammatory cytokines. The chemokines that are produced in inflammation vary from one tissue to another, and also depend on the type of infection, damage and immune response that is taking place.

- ☐ We noted above that different types of leukocytes are characteristic of different types of inflammation. How can chemokines selectively attract one population of cells into an area?
- ☒ Only a cell that has receptors for that specific chemokine will be able to respond to it. As noted above, different populations of leukocytes express distinct sets of chemokine receptors.

In addition to the chemokines, there are a variety of other chemotactic molecules (Table 2.1). You have already encountered the complement fragment C5a, and this works in a very similar way to the chemokines, attracting monocytes and neutrophils to a site of inflammation. Other important chemotactic molecules are bacterial peptides. Bacteria initiate their protein synthesis using a special amino acid formylmethionine (fMet) and there are receptors on phagocytes for peptides that have this amino acid. It means that if a bacterial infection is present, phagocytes will automatically be attracted to it. Much more is known about the way in which leukocyte traffic is controlled, but you do not need to know the detail.

TABLE 2.1 Examples of chemotactic molecules.

Mediator	Source	Targets
C5a	complement system	neutrophils, monocytes, macrophages
fMet.peptides	bacteria	neutrophils, monocytes, macrophages
leukotriene B4 (LTB4)	mast cells macrophages	macrophages, some lymphocytes
chemokines	leukocytes tissue cells	lymphocytes, mononuclear phagocytes, granulocytes

2.3.4 Processes of inflammation



Following this introduction to the subject, you should now work through the topic on CD1 entitled *Inflammation* that is accessed via the Navihedron icon of a blood vessel. As you go through this topic, you will be introduced to some of the ideas above. Be sure to see the videos of lymphocyte migration under: 'Migration of lymphocytes across endothelium in vitro' and 'Interactions of lymphocytes with endothelium in vivo'. Also you should see the information on the screens: 'Mediators of inflammation' and 'The immune system in acute inflammation'. These are all accessed from the expansion lists linked to the *Inflammation* animation.

After completing this topic, you should be able to answer Questions 1, 2, 3, 4, 8 and 10 from this section. The descriptions of inflammation in the animation have highlighted some of the mediators that control the process and the following text expands on this area.

You should spend about one hour on this activity.

Mast cell mediators

In addition to their role in promoting leukocyte traffic, inflammatory mediators control other aspects of inflammation too. Mast cells are very important sources of mediators, since they have granules containing chemotactic molecules and histamine, which acts on blood vessels to increase blood flow and capillary permeability. In addition, if they are activated, mast cells synthesize a group of molecules called **eicosanoids** (a group of small molecules derived from fatty acids via arachidonic acid), some of which modulate the local blood supply, while others are chemotactic and others can mediate sensations of pain.

The kinin system

Another important group of mediators is produced by the **kinin system**. Like complement, this is a plasma enzyme system, but its main function is in controlling blood supply and capillary permeability. The kinin system is activated when damage occurs in the tissue and not only when there is infection. It can also be activated in association with other plasma enzyme systems. One of the mediators of this system, bradykinin, is extremely potent at causing dilation of blood vessels and enhancing permeability. The ways in which the systems interact with each other in inflammation is outlined in Figure 2.14.

2.3.5 Inflammation and disease symptoms

There are a number of questions which have probably occurred to you even before starting this course, concerned with your own experience of infections:

- Why when we have an infection do we feel ill?
- What advantage is there in feeling awful when we have an infection?
- What mechanisms lead to sickness behaviour?

These simple questions are difficult to answer. Although toxic molecules produced by pathogens can have effects within the body, symptoms of the disease (e.g. fever) are not generally created by the pathogens themselves. The reason that we address these questions here is because some of the mechanisms can be ascribed to

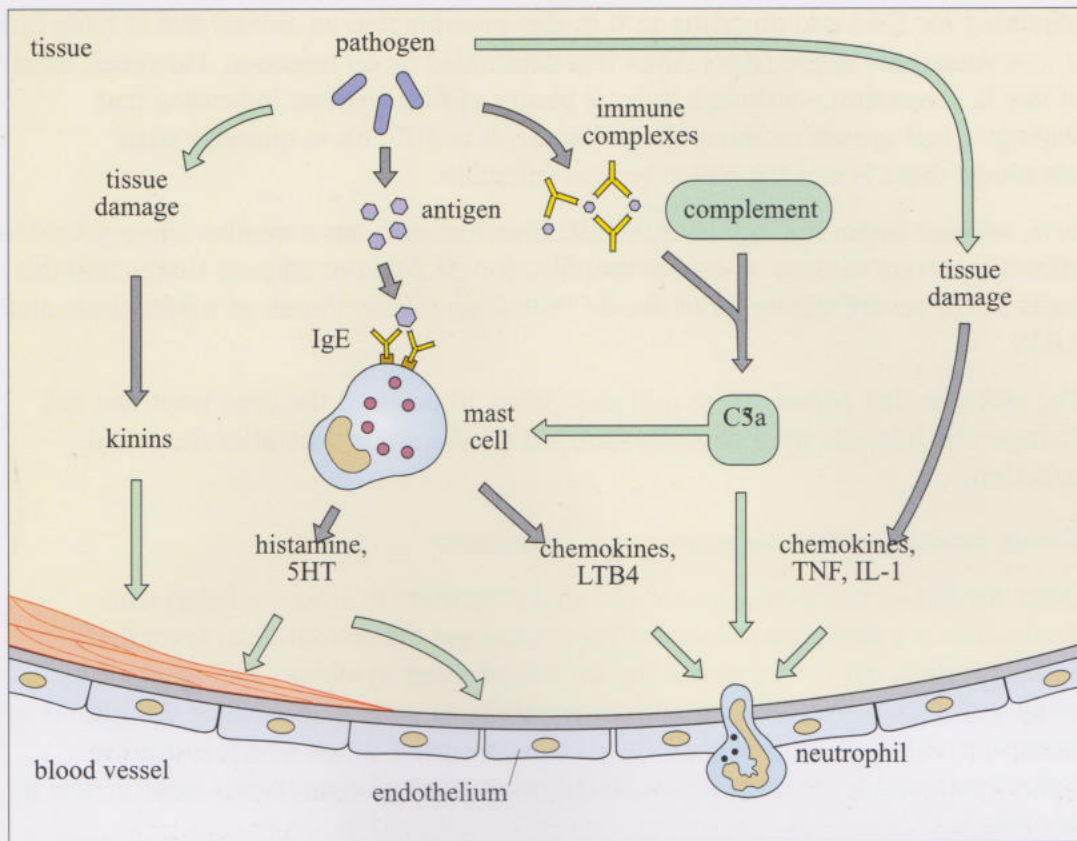


FIGURE 2. 14

A summary of the processes involved in inflammation. Pathogens cause tissue damage, which activates the kinin system. Mediators of this system act on blood vessels to increase blood flow and enhance capillary permeability. Antigens from the pathogens can also bind to IgE antibodies on the surface of mast cells, triggering the release of histamine and 5-hydroxytryptamine (5HT) which also enhance blood flow and permeability. Immune complexes formed by antigens and IgG or IgM antibodies can activate the complement system resulting in the production of C3a and C5a which also trigger mast cell degranulation. Chemokines from the mast cells or produced by other cells in the tissue in response to damage, leukotriene B₄ (LTB₄) an eicosanoid, complement C5a and inflammatory cytokines (TNF α and IL-1) all contribute to the accumulation of leukocytes at the site of inflammation.

the actions of inflammatory mediators. First we should identify some of the characteristics of sickness:

- fever
- aches and pains
- loss of appetite
- tiredness and muscle weakness

The role of cytokines in sickness

An important mediator of many of the symptoms is IL-1. This cytokine was identified in one of its many guises as 'endogenous pyrogen', that is, an internal molecule which induces fever. Injection of IL-1 into experimental animals causes an increase in their temperature, by action on temperature regulation centres in an area of the brain called the hypothalamus. IL-1 may act via another mediator IL-6, which is also involved in the more general response to infection. One consequence of a raised temperature is that it causes lymphocytes to divide more rapidly, but it has only a marginal effect on the rate of pathogen division. Since the development of sufficient lymphocytes in the early phases of infection is critical in the race against the pathogen, we can presume that one advantage of fever is to give the immune system an edge in this race.

IL-1 injection also induces loss of appetite and increases the amount of 'slow wave' sleep that an individual takes. Together with the pain, tiredness and weakness that are associated with disease, these symptoms conspire to reduce mobility. There are some clear advantages to having reduced mobility. Physically, it means that pathogens are less likely to spread through the body tissues; it is advantageous to confine infection as much as possible. Second, the reduced activity allows a greater amount of available energy to be diverted for immune defence, and less to

searching for food and digesting it. It is also possible that an animal that is lying low is less vulnerable to predators when it is debilitated by an infection. However, most of this is conjecture – although there is plenty of folk wisdom indicating that staying in bed speeds recovery from disease, it is difficult to quantify what advantage there is and for which type of infection.

TNF, another important mediator of inflammation, also has a number of very visible effects in severe disease. It causes mobilization of fat from adipose tissue, and this leads to the severe wasting that occurs in serious diseases such as tuberculosis and AIDS.

The sickness that accompanies a disease tends to occur at the time when the cell damage and immune responses are most active, i.e. some time after the initial infection.

Kinins, prostaglandins and neurogenic components

Other mediators mentioned above can also contribute to sickness behaviour. Bradykinin is a powerful inducer of pain. Amongst the eicosanoids, several of the prostaglandins, act to accentuate the actions of other cytokines and other mediators. For example they increase sensitivity to pain, and enhance the actions of histamine and bradykinin. One of the major groups of drugs which is used to control pain and fever interferes with the production of eicosanoids (aspirin acts in this way).

The examples given are probably important when severe generalized infections occur, but more subtle effects may occur as a result of local release of mediators acting directly on neurons. For example, the stress-related hormone corticotropin-releasing factor modulates the firing rate of neurons in part of the mesolimbic system, an area of the brain involved in feeling and emotion.

2.4 Antibodies and complement in defence against bacteria

Anyone who has a genetic deficiency which reduces their ability to make antibodies or complement molecules becomes more susceptible to infection with bacteria. This tells us that antibodies and complement are important in defence against such infections.

- ☐ Would you expect such individuals to be more susceptible to all bacterial infections? And if it is only some infections, which ones?
- ☒ It tends to be infections with those bacteria which normally live outside cells, for example *Staphylococcus*, *Streptococcus*, *Haemophilus* – not surprising really, as we noted earlier that antibody was a key component of defence against extracellular pathogens.

2.4.1 Neutralising antibodies

Antibody provides a specific way of recognising bacterial molecules, and most importantly, it can direct complement activation onto the bacterial surface via the classical pathway. So let us consider some ways in which antibodies and complement can protect against bacterial infection.

The first is by simple neutralization of toxic molecules. Many bacteria produce their damage by releasing toxins that move through the blood. Examples are the toxins from tetanus and diphtheria or the toxic molecules produced by some organisms which cause food poisoning (e.g. botulism). If antibody binds to these molecules, it can often neutralize them completely and high affinity antibodies are most effective at neutralization.

- ☐ What class of antibody is likely to be most effective in countering such toxins?
- ☒ IgG, the main serum antibody, prevents toxins spreading through the blood. Also, IgG antibodies are generally of higher affinity than IgM.

Many bacteria produce their damage by invading tissues, while producing enzymes and toxins that allow spreading and dispersal. Antibodies against these components are also important in limiting spread. An antibody that is bound to the active site of an enzyme will prevent it from working properly. Even antibodies that bind to other sites of the enzyme may affect its function and will promote the formation of immune complexes which can be phagocytosed and broken down by neutrophils and macrophages. Antibodies against flagellae or related components often reduce bacterial motility and thus limit spread of the bacteria through the body.

2.4.2 Actions of IgA

When considering how bacteria invade the body we noted that IgA protects the mucous membranes and prevents bacteria from attaching to these surfaces. B cells that produce IgA tend to localize in MALT, but the antibody they produce needs to be transported across the epithelium to reach the mucosal surface (Figure 2.15). The process is very efficient. For example, the concentration of IgA in blood is 2 mg/ml while the concentration in tears is 16 mg/ml. But notice that serum IgA and

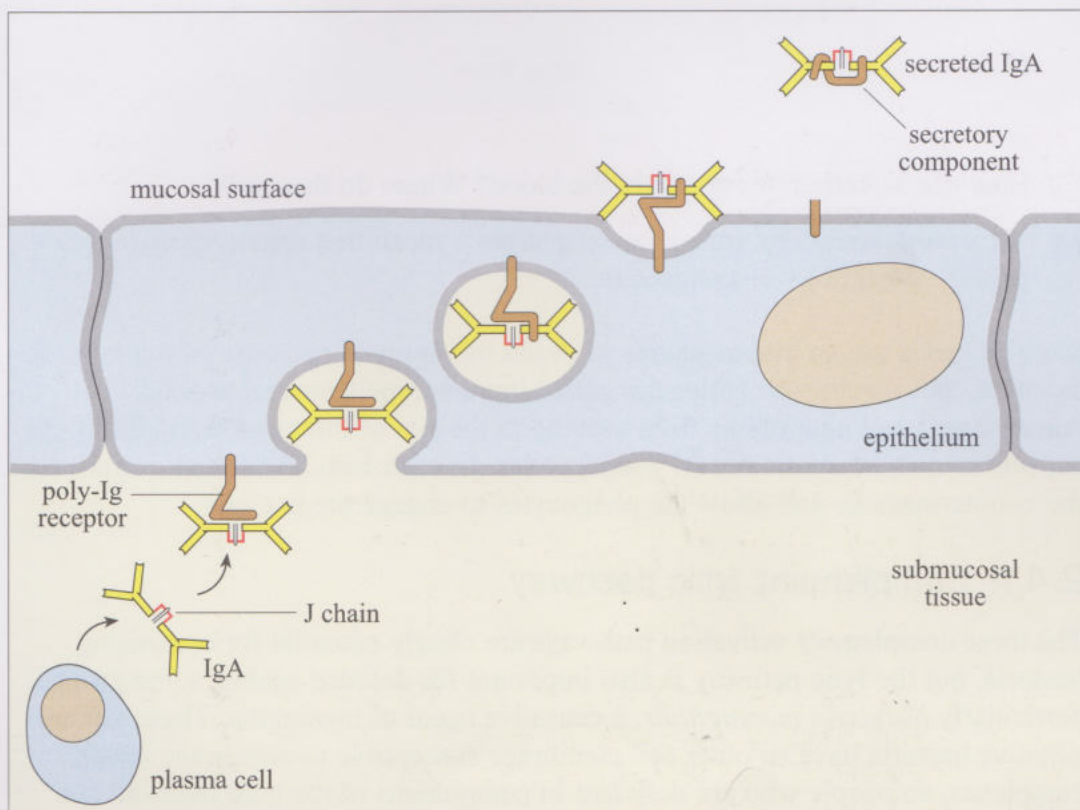


FIGURE 2.15

IgA is produced by plasma cells in the submucosal tissue and becomes bound to the poly-Ig receptor on the tissue side of epithelial cells. The bound IgA is transported across the epithelium in vesicles and is released at the mucosal surface, by cleavage of the poly-Ig receptor. The segment of the receptor that remains bound to the secreted IgA is the secretory component, which helps prevent degradation of the Ig by enzymes.

secretory IgA are slightly different: the latter has the secretory piece attached as it is transported across the epithelium and this polypeptide tends to prevent the IgA from being degraded by enzymes in the secretions (e.g. digestive enzymes). When devising vaccines to combat infections which enter through mucous membranes it is usually desirable to induce good IgA responses, and this is done by promoting a local immune response at the site of entry, rather than a general immune response. Antibodies to fimbriae, which some bacteria use to attach initially to a host cell, are particularly important targets for this type of defence.

2.4.3 Opsonization of bacteria

If bacteria enter the body, clearance is dependent on phagocytosis, and the efficiency of the phagocytes often depends on both antibody and complement which are needed to opsonize the bacteria (Figure 2.16).

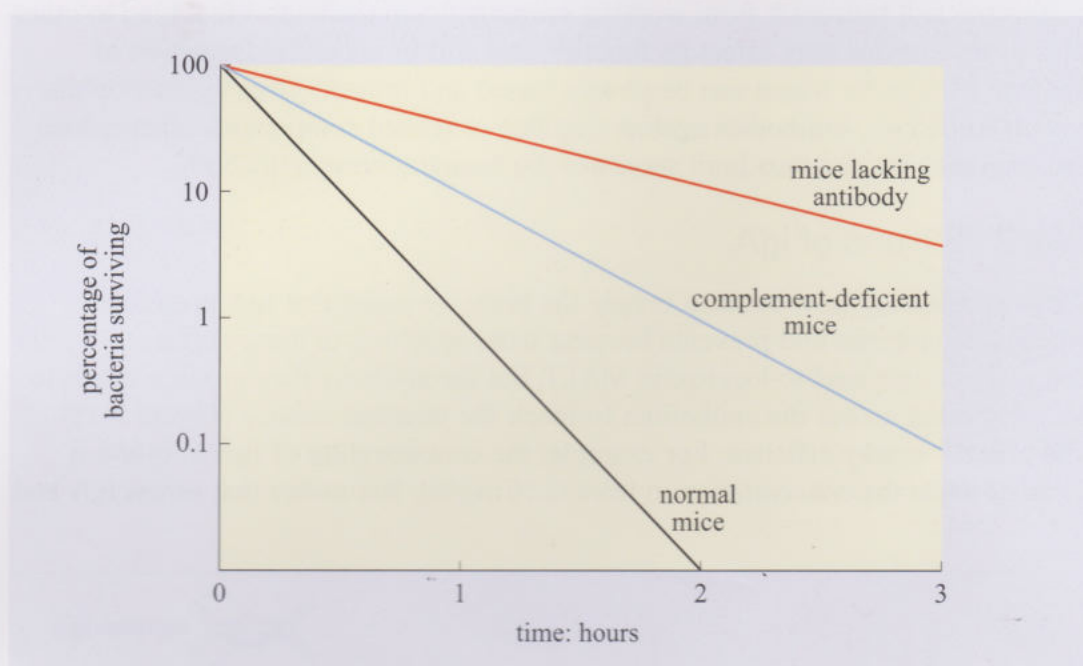


FIGURE 2.16

The rate of clearance of a known dose of bacteria from the blood depends on antibody and complement. In normal mice with antibody against the bacteria, 99% of the bacteria are cleared within one hour. If the animals are temporarily depleted of complement (by an experimental manipulation) clearance is slower, and it is slower still if the animals do not have antibodies against the bacteria.

- ☐ How are bacteria removed from the blood? Where do they go?
- ☒ They are taken up by splenic macrophages – recall that splenectomy leaves a person susceptible to septicaemia.

Many bacteria use countermeasures to evade the immune response of the host. For example, they can release molecules called immunorepellents that prevent macrophages and neutrophils from moving to the site of infection. In addition, the capsule of many bacteria resists phagocytosis. In each case, antibodies neutralize the countermeasure and allow the phagocytes to engage the bacteria.

2.4.4 Complement lytic pathway

The three complement activation pathways are clearly essential for opsonising bacteria, but the lytic pathway is also important for defence against some groups, particularly *Neisseria meningitidis*, a causative agent of meningitis. These Gram-negative bacteria have an outer cell membrane susceptible to membrane attack complexes, so people who are deficient in components of the lytic pathway are more susceptible to this disease.

Summary of Sections 2.1–2.4

- 1 Antibodies specifically bind to antigens on a pathogen and may interfere with its ability to attach to and infect host cells. Antibodies can also act as adapter molecules allowing cells of the body (e.g. phagocytes) to bind to antigen.
- 2 Different classes of antibody have different functions. IgA is secreted across mucosal membranes, and is important in protecting these sites against pathogen entry. IgG and IgM antibodies promote phagocytosis, either directly, or by activating the complement system. IgE is important in the development of inflammation.
- 3 Antibodies bind to epitopes using multiple non-covalent bonds. The binding strength determines the affinity of the antibody.
- 4 The complement system is a group of molecules, present in blood and tissue fluids which can be activated either by immune complexes or by the surface of many pathogens, and which lead to pathogen destruction and/or the induction of inflammation.
- 5 Inflammation is a process that brings cells and molecules of the immune system to tissues at sites of infection or cell damage.
- 6 Cytokines released by leukocytes and mediators produced by mast cells and the kinin system also modulate inflammation.
- 7 Many of the symptoms of disease are due to the actions of inflammatory cytokines. Cytokines such as IL-1 and TNF are responsible for some disease symptoms, such as fever and appetite loss.
- 8 Antibodies and complement protect against bacteria, by neutralizing bacterial toxins and enzymes, by preventing bacterial adhesion, by directly damaging the bacterial cell membrane and by promoting their phagocytosis.

2.5 Phagocytes and phagocytosis

Ultimately, opsonized bacteria, viruses, unicellular parasites and immune complexes are taken up for destruction by phagocytes. These may be the neutrophils which are mobilized to areas of acute infection and inflammation. It could be the fixed mononuclear phagocytes that reside in the tissue or the mobile macrophages that are recruited during infection. Typically, macrophages are more prevalent at sites of *chronic* infection. These cells are very well equipped to deal with pathogens; they contain an arsenal of molecular killing systems that they can turn onto pathogens they have phagocytosed. Mononuclear phagocytes also secrete a large number of molecules, including cytokines, which bolster the local immune defences. These cells do not work in isolation: in particular macrophages are very much dependent on TH1 cells for recognition of the pathogen and require cytokines released by TH1 cells in order to develop their full range of effector functions – a process which is called **macrophage activation**.

- ☐ Apart from the release of cytokines, what direct cellular interactions take place between the TH1 cells and macrophages?
- ☒ Macrophages present antigen to TH1 cells (see Section 1.2.2).

In the discussion below we will consider the functions of neutrophils and macrophages together, since they share many of them. However, macrophages as long-lived cells carry out many functions that neutrophils do not, and neutrophils have one or two special antibacterial defences that macrophages do not possess, and these will be highlighted at the appropriate point. We will consider in sequence the processes that occur as a macrophage moves to a site of infection to phagocytose and destroy a pathogen.

2.5.1 Chemotaxis and cell migration

Monocytes and macrophages have a variety of receptors which allow them to respond to chemotactic stimuli, including a receptor for C5a, one for fMet-peptides, at least five different receptors for chemokines and a receptor for an eicosanoid leukotriene-B₄ (LTB₄). This last product is a small molecule that is produced by mast cells that have been activated. The receptors allow the macrophage to respond to a wide range of stimuli. In practice the cell responds to several different stimuli. First it needs a trigger to cross the endothelium, and then it needs further signals to move to the site of infection. It is rather like orienteering, with the cell moving from one place to another and getting new directions for its next destination.

The cell uses integrins on its surface to allow it to bind, first to the endothelium and later to molecules in the extracellular matrix (e.g. collagen or laminin). (Integrins belong to a large family of molecules involved in adhesion. We have already mentioned some integrins that are involved in binding of leukocytes to cell adhesion molecules on the endothelium, during migration across the endothelium; Figure 2.13.) Integrins are attached to the cytoskeleton of the cell, and this allows the cell to move through tissues. The basic mechanisms of cell migration are outside the scope of this course, but have been explained in the Open University course S204, *'The Core of Life'*, Chapter 7, which can be accessed on the S320 Reference CD-ROM.

2.5.2 Phagocytosis

The first step in phagocytosis is binding to the pathogen which is to be internalized (Figure 2.17). We have already seen how important antibody is in this process, and macrophages have two types of receptor for IgG, FcγR1 and FcγRIII. They also have three different types of receptor for activated complement. The **Complement Receptors** that are present on macrophages are designated CR1, CR3 and CR4, and these allow the cell to bind to anything with C3 fragments bound to the surface. (Incidentally, two of these molecules, CR3 and CR4, are integrins). The receptors allow the cell to target anything that has been identified by antibody or complement, and when they bind to targets they promote phagocytosis and activation of the killing mechanisms described below.

In addition, macrophages have a number of receptors that recognize generic structures found on microbial pathogens. The term 'pattern recognition' has been used to describe these receptors which recognize a variety of non-self molecules. Many of the receptors bind to complex carbohydrates, such as those found in bacterial and fungal cell walls.

An example of such a receptor is the mannose receptor (MR), which has eight domains belonging to a family of lectins (lectins are carbohydrate-binding

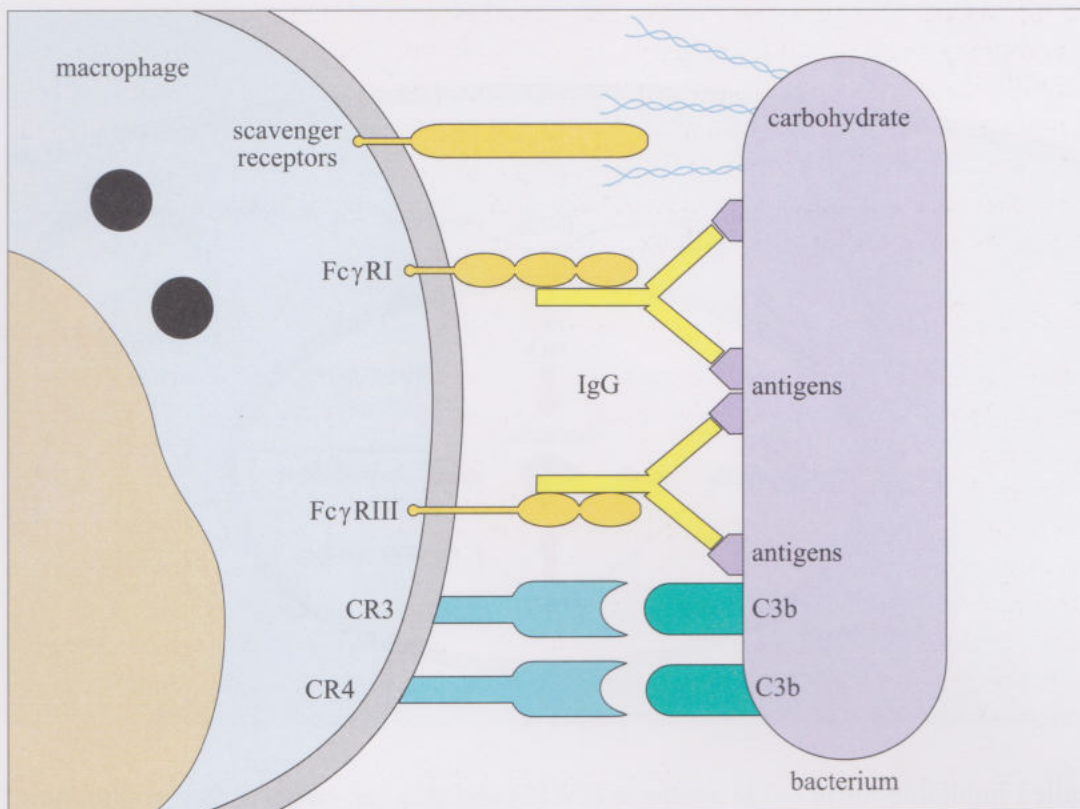


FIGURE 2.17 Macrophages use a variety of receptors to recognize bacteria. This may be direct recognition of bacterial surface components such as carbohydrates, or may be indirect recognition by adapter molecules such as IgG or C3b.

molecules). In some cases receptors on macrophages also bind to self components, and these appear to be involved in clearing up host cell debris released when cells die, and are not just involved in recognising pathogens. A lot of cell death takes place in the body as part of normal everyday life, and cell death also occurs as a result of infection. Some of the receptors involved in this clearing-up process belong to the family termed 'scavenger receptors'.

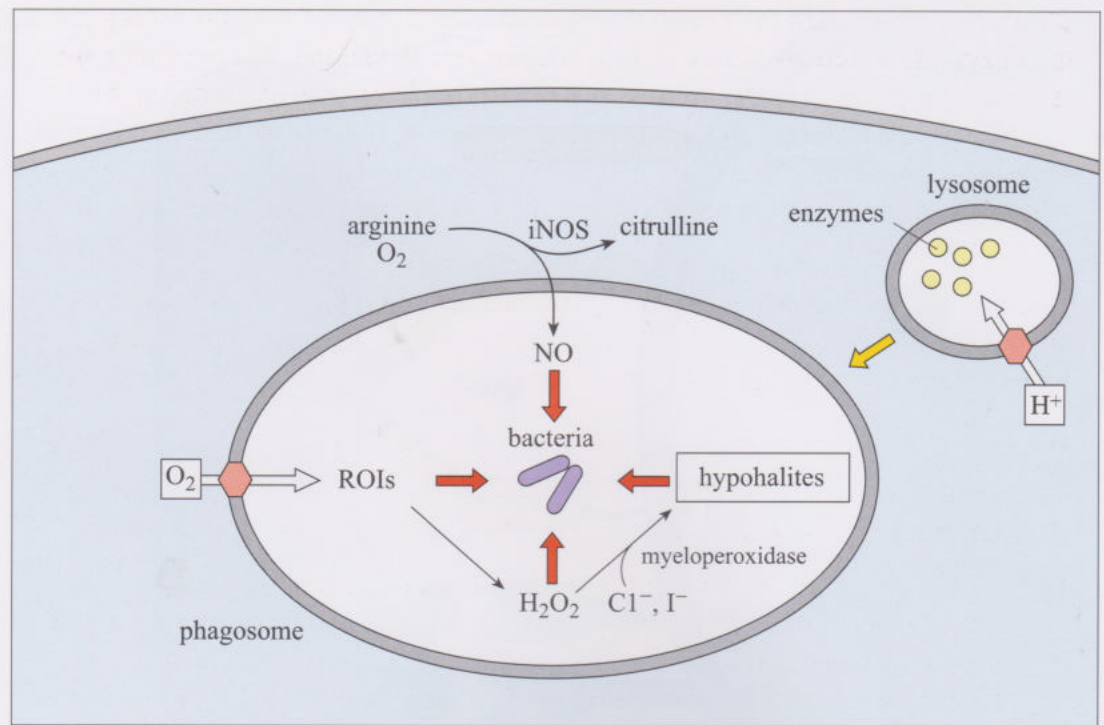
2.5.3 Killing mechanisms

Once the pathogen has bound to the receptors, pseudopods extend around it, so that it becomes enclosed in a **phagosome** – a membrane-bound vesicle, which contains phagocytosed material. The macrophage will now direct its biochemical arsenal towards the phagosome, where the pathogen is contained (Figure 2.18). In the first instance the cell assembles an enzyme in the phagosome membrane which pumps electrons into the phagosome, thereby generating highly reactive superoxide anions. These can go on to generate further reactive molecules including hydrogen peroxide, and hypochlorite (bleach). The collection of molecules is collectively termed **reactive oxygen intermediates (ROIs)**. They are very toxic for pathogens. Indeed, you will probably have noticed that these are the same kind of molecules that we use out of a bottle when we want to kill bacteria. The macrophage just got there first.

Macrophages that have been activated by the inflammatory cytokines $\text{IFN}\gamma$ and $\text{TNF}\alpha$ can generate a separate group of toxic molecule **reactive nitrogen intermediates (RNIs)**. When the macrophage is activated it synthesizes an enzyme

FIGURE 2.18

Macrophages use a variety of mechanisms to kill bacteria located in their phagosomes. Enzymes in the phagosome membrane generate reactive oxygen intermediates (ROIs) which may become converted to hydrogen peroxide and hypohalites (e.g. hypochlorite), all of which are toxic to certain pathogens. Nitric oxide (NO) formed by the action of inducible nitric oxide synthase (iNOS) on arginine is also toxic, especially when it combines with ROIs. Lysosomes containing enzymes are maintained at a low pH by proton pumps in the lysosome membrane. After the lysosome fuses with the phagosome, the enzymes contribute to the degradation of the killed bacteria.



called inducible nitric oxide synthase (iNOS) and this can catalyse the production of nitric oxide, another highly reactive and somewhat toxic molecule. Combination of the ROIs and nitric oxide produces another batch of toxic compounds, the peroxynitrites, which are toxic for fungi, and many other eukaryotic parasites and bacteria.

- ☐ Why is the macrophage itself not damaged by the biochemical attack on phagocytosed pathogens?
- ☒ The toxic molecules are confined to the phagosome, which is isolated from the cytoplasm and cellular organelles by the phagosome membrane. Additionally, the macrophage has a limited capacity to mop up reactive intermediates that leak out of the phagosome.

This chemical barrage is just the first phase of the attack. Immediately after the phagosome has formed, the pH inside it starts to rise. This can partly be attributed to hydroxyl ions produced as part of the process above. Later on the pH will fall and the phagosome becomes acid. During the alkaline phase a new defence comes into action. Neutrophils (but not macrophages) have a group of cationic proteins called **defensins** in their granules that are released into the phagosome. They have a wide spectrum of activity against Gram-positive and Gram-negative bacteria, spirochaetes and yeast, including the ability to damage the outer membrane of the pathogens by assembling ion channels in them.

- ☐ Can you recall any other instances when pathogens are damaged by the assembly of channels in their outer membranes?
- ☒ The membrane attack complex of the complement lytic pathway produces channels through viral envelopes and the outer membranes of some bacteria.

In the final phase, the phagosome becomes acidic and degradative enzymes are released into the phagosome to break down the pathogen, which by this stage is

(hopefully) dead. Just the process of acidification is toxic for some pathogens, but the enzymes, which are active at low pH, are also damaging. The enzymes are normally held in lysosomes, which fuse with the phagosome, to produce a combined vesicle called a **phagolysosome**. The phagolysosome can also contain growth inhibitors. For example, neutrophils produce a molecule called lactoferrin, which binds avidly to iron. Since bacteria and parasites need iron to grow, its removal by binding to lactoferrin prevents them getting the supply they need to divide.

You can see that a phagolysosome is not a comfortable place for a microorganism, and all persistent pathogens have evolved countermeasures that allow them to deflect some of the phagocytes' defences.

- ☐ If a pathogen had managed to enter the cytoplasm of the macrophage, either because it had infected the cell directly or because it had escaped from the phagosome, do you think that the macrophage would still be able to kill it?
- ☒ No, the only way the macrophage can let loose its chemical arsenal is by confining everything to the phagolysosome. If a pathogen escapes into the cytoplasm, the macrophage is now an infected cell and the pathogen can replicate inside it. This is what occurs in tuberculosis and with a number of other pathogens that live inside macrophages.

2.5.4 Antigen presentation

The breakdown of the pathogen is not the end of the story. Remember that the macrophage is also an antigen-presenting cell. Peptide fragments can be transported through the cell to become combined with MHC molecules, which are then presented to T_H1 cells. We shall be considering antigen presentation in detail later, but at this stage it is important to know that an antigen-presenting cell, like a macrophage sends several signals to a T cell. There is the antigen peptide on the MHC molecule, but equally important are the signals that go alongside this, which are called **costimulatory signals** (Figure 2.19).

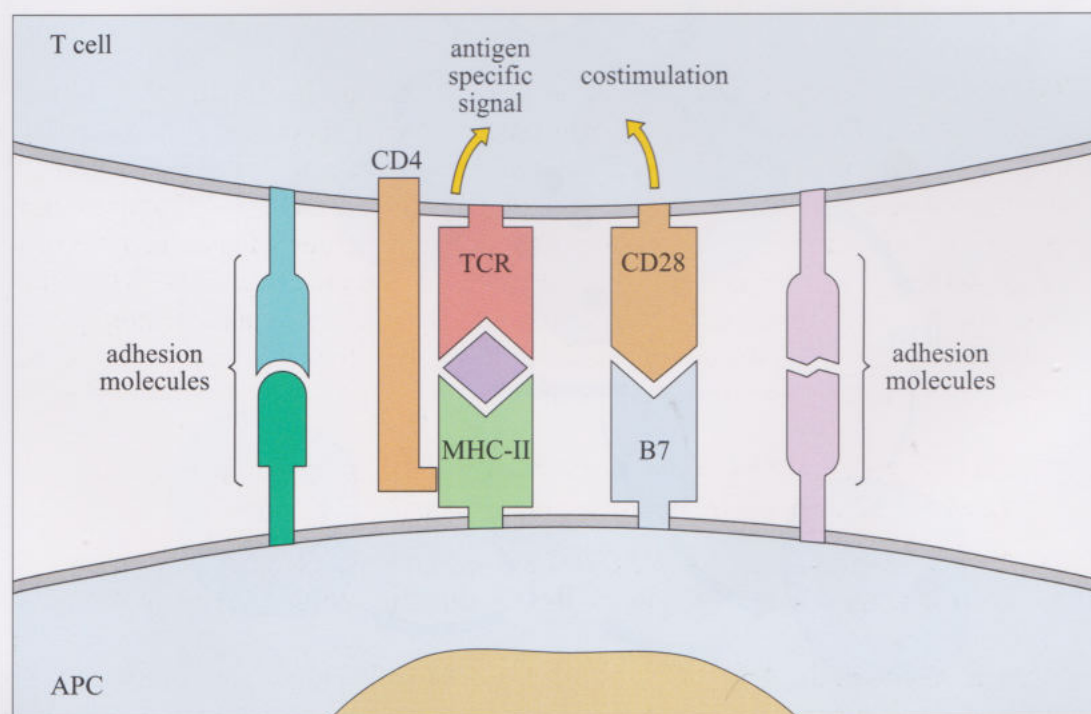


FIGURE 2.19
Antigen-presenting cells (APC) initially bind to T cells using adhesion molecules. This allows the APC to present antigenic peptides bound on its MHC class II molecules. The T cell recognizes this complex using its T cell receptor (TCR) and CD4 molecule. This gives a signal to the T cell. However, the T cell also requires costimulation transduced by molecules such as CD28, if it is to become activated.

How does a macrophage 'know' when to send these costimulatory signals? It turns out that the molecules that send the signals are induced by bacterial products. For example, macrophages have on their surface receptors for lipopolysaccharide (LPS), which is a component of the cell wall of Gram-negative bacteria. When it detects this bacterial component, the macrophage will increase the expression of its costimulatory molecules and thus become a more effective antigen-presenting cell.

Interestingly, the macrophage appears to have a number of such systems for recognising bacterial products, which appear to be very ancient in an evolutionary sense. This reflects the fact that macrophages have been present throughout the evolutionary history of animals, and were present long before the evolution of lymphocytes and the more advanced types of immune recognition. The evolution of antibodies and Fc receptors has given the macrophage just one more way of recognising pathogens.

2.5.5 Cytokines and secreted molecules

Macrophages produce and respond to a number of cytokines (Figure 2.20). Most importantly, they are activated by $\text{IFN}\gamma$, released by antigen-stimulated Th1 cells. This cytokine was once described as macrophage-arming factor, which describes

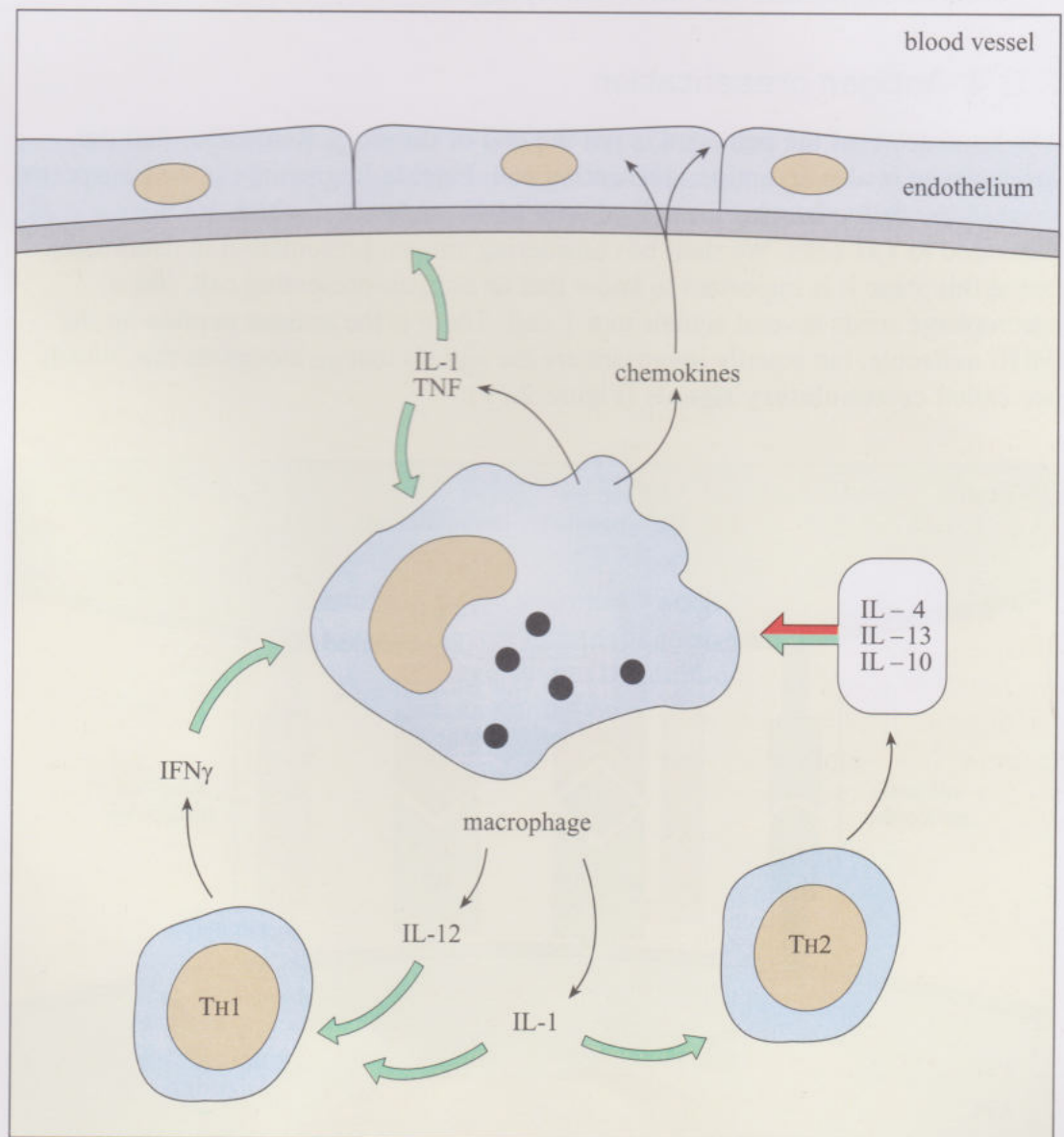


FIGURE 2.20
The macrophage is central to immune responses. Chemokines and the cytokines TNF and IL-1 act on endothelium to promote leukocyte migration into tissue. Macrophages are activated by $\text{IFN}\gamma$ and TNF to promote microbicidal activity. They are regulated by cytokines from Th2 cells including IL-4, IL-13 and IL-10. They also produce IL-12 which promotes production and activation of Th1 cells and IL-1 which enhances proliferation of T cells.

its function very well. $\text{IFN}\gamma$ enhances all of the macrophage's killing mechanisms, and since the macrophage can present antigen to the T cells, there is a positive feedback loop here. If the T cell recognizes something that the macrophage has taken up and is presenting on its surface, then it will release $\text{IFN}\gamma$, which in turn enhances the ability of the macrophage to destroy it. Cooperative recognition and activation is crucial in defence against pathogens because there are many pathogens that can survive in macrophages that have not been activated, but which are killed by activated macrophages.

$\text{IFN}\gamma$ not only enhances the ability of macrophages to kill pathogens, it also increases their ability to present antigen to the T cells. It does this by inducing the synthesis of MHC molecules and costimulatory molecules. So here we have another cooperative interaction between the Th1 cells and the macrophages.

Macrophages themselves produce cytokines when they have been activated, including tumour necrosis factor- α and interleukin-12 (IL-12). You have already encountered $\text{TNF}\alpha$ as a cytokine that induces adhesion molecules on endothelium and thereby promotes cell migration into sites of infection. Related to this, mononuclear phagocytes are important sources of the chemokines which are needed to trigger migration of additional leukocytes to the inflamed tissue. Many cells of the body can respond to $\text{TNF}\alpha$, including the macrophages themselves. This kind of response, where a cell produces a cytokine that acts on itself and similar cells in the area, is called an autocrine action. It means that an activated macrophage can signal to neighbouring cells to enhance their killing mechanisms too.

The cytokine IL-12 is very important in immune responses mediated by macrophages. It acts to promote the development of further Th1 cells. You can see here yet another way in which the macrophages and Th1 cells reinforce the action of each other. This is such a central feature of immune responses that immunologists have come to call responses involving interacting Th1 cells and macrophages a Th1 -type response. In practice, it corresponds closely to what has been traditionally called a cell-mediated immune reaction. We must return later to the subject of different types of immune reactions, since you will recall that different types of immune reaction are appropriate for some pathogens but inappropriate for others.

Macrophages secrete many other molecules, in addition to the cytokines. For example, macrophages can produce complement system molecules, including C3. We should mention here that most of the complement system molecules are synthesized by the liver, but the ability of macrophages to produce them is extremely useful. Complement molecules will be used up at sites of infection, and we know that complement is necessary for macrophages to phagocytose bacteria. It is therefore handy that macrophages are able to produce complement to top up the local levels of these mediators.

- ☐ Can you recall an example of a complement component which macrophages can recognize and how they recognize it?
- ☒ They have receptors CR1, CR3 and CR4, which allow them to recognize bound C3 fragments (Figure 2.17). Intact C3 is produced by activated macrophages.

Another important molecule secreted by macrophages is the enzyme lysozyme, which is able to cleave peptidoglycans in the cell walls of bacteria. Macrophages constitutively produce lysozyme, and it is found in many secretions. If the cell wall of a bacterium is damaged the bacterium may burst by osmotic shock, and this allows other components of the immune system access to the cell membrane.

- ☐ Is there any way in which lysozyme could damage Gram-negative bacteria, which have an outer plasma membrane shielding their cell wall?
- ☒ Lysozyme cannot normally access the cell wall of Gram-negative bacteria, but if the complement lytic pathway damages the outer membrane, then it allows lysozyme to access the cell wall (see Section 2.3.1). So complement and lysozyme work in concert.

2.5.6 Overview of cell migration and phagocytosis



Now you have an understanding of the functions of phagocytes you should work your way through the topic on CD1 entitled *Cell migration and phagocytosis*, which is accessed from the Navihedron icon of a moving macrophage. As you work through it make sure you see the video page of 'Chemotaxis and Phagocytosis' which dramatically demonstrates the ability of macrophages to hunt down and phagocytose yeast cells.

You should also see the expansion screens entitled 'Phagocytosis', 'Phagosomes and lysosomes', 'Bacterial killing by phagocytes' and 'Bacterial evasion of phagocytes'. The screen entitled 'Recognition of bacteria by phagocytes' makes a good final summary of this area.

This section on the CD-ROM is intended to reinforce your understanding of the role of phagocytes in immune defence. Once you have finished this topic, you should be able to answer all of the self-assessment questions that are associated with it.

You should spend about one hour on this activity.

2.6 The role of mononuclear phagocytes in bacterial infections

You should now appreciate the importance of mononuclear phagocytes and macrophages in defence against bacterial infections, particularly those that produce acute infections.

- ☐ Children are sometimes born with genetic defects that affect the ability of their macrophages to produce reactive oxygen intermediates. What effects do you think this would have on their ability to fight infection?
- ☒ Children with these defects suffer from serious bacterial infections, particularly with streptococci, staphylococci, pneumococci and haemophilus. This results in pneumonia, infections of the lymph nodes and abscesses in the skin and internal organs. The range of infections is similar to that seen in patients who lack antibodies.

These observations show us that macrophages are required to control these acute infections. The fact that most people are not greatly troubled by such infections shows that macrophages are normally very effective in this aspect of antibacterial defence. However, there are a group of chronic infections in which macrophages are equally important, these include infections such as tuberculosis, leprosy, typhoid, and shigellosis. In each of these infections, macrophage-mediated immunity is an important component of protection, but the macrophage is also the cell that becomes infected by the bacteria. In order to do this and survive these groups of bacteria deploy a variety of measures designed to evade the macrophages' defence mechanisms. In this case, the effectiveness (or otherwise) of the immune response often depends on whether the macrophage has been activated by a pathogen-specific T cell.

Let us consider some examples of these types of bacteria. As we do so, notice that in each case the chronic infection occurs because macrophages become infected and are unable to eliminate the bacteria completely.

2.6.1 Typhoid

Typhoid is a disease caused by *Salmonella typhi*, which is contracted from contaminated water. It affects up to 12 million people per year, and mortality can be as high as 30% if people do not receive adequate treatment, but as low as 1% with good treatment, including the early use of antibiotics. The bacteria enter the body from the gut by crossing the cells that overlie the Peyer's patches. This gives them direct access to cells of the immune system, and from there they can spread throughout the body, to infect mononuclear phagocytes in many different tissues. The symptoms, which develop after 1–3 weeks, include fever, severe abdominal cramps, diarrhoea, and symptoms such as muscle pains and dizziness. Serious developments such as intestinal ulceration and bleeding produce the mortality.

Because *S. typhi* does not produce disease in other animals, much of our knowledge comes from the related mouse disease caused by *Salmonella typhimurium*. Control of this infection depends on macrophages and the bacteria are susceptible to cationic proteins and the oxygen-dependent killing mechanisms (ROIs), but they have a mechanism to delay lysosomes and granules from fusing with the phagosome, which limits their exposure to the toxic molecules. If the bacteria survive for a few hours inside the macrophage, they adapt to the intracellular environment, expressing a range of proteins that limit the damage caused by the macrophage and can survive and replicate at a low level. Survival of the bacteria is therefore partly dependent on whether the macrophages are activated at an early stage of infection, and this depends on IFN γ and TNF α . Treatment of macrophages with IFN γ in vitro increases the rate at which lysosomes fuse with phagosomes containing *Salmonella*. Conversely, inhibiting these cytokines makes the animals much more susceptible to infection. The cell-mediated response against *Salmonella* is clearly important, but antibodies are also required, to allow the bacteria to be opsonized. For example, it is possible to protect mice against infection by transferring into them both antigen-specific T cells and antibacterial antibody – the T cells activate the macrophages and the antibodies allow the macrophages to recognize the bacteria.

One more important feature of typhoid should be noted: a proportion of individuals who recover from typhoid, and a small number who never exhibit the disease, act as chronic carriers of the bacteria for many years. Their macrophages suffer from

a permanent low-level of infection. The carriers have high levels of immunity, including specific antibodies (against salmonella), but they continue to shed the bacteria in faeces. From the viewpoint of the bacteria, this is ideal – a carrier who does not appear to be ill, continues life as normal and can continuously contaminate food or water with the bacteria.

2.6.2 Tuberculosis mini-lecture

The mycobacteria that produce tuberculosis and leprosy also survive in macrophages. This group of pathogens has particularly tough cell walls that allow them to survive indefinitely within macrophages, where they can divide slowly. Immunity mediated by TH1 cells is again particularly important, and one defence used by the bacteria is to interfere with the signalling systems inside the macrophage, so that they cannot respond to IFN γ . Different ways in which pathogens counter host defences are examined later in the course, but it is worth noting here that the countermeasures which a bacteria uses are selectively directed at just those immune defences which are directed against the bacteria.



At this stage you should view the mini-lecture on tuberculosis on CD2. This will consolidate your understanding of tuberculosis from the case study. It also explains to you the immune defences that the body directs against mycobacteria, and it introduces a number of new ideas that we shall take up later. In particular, notice how the bacteria are very widespread, but the incidence of the disease depends on many factors, including the genetic make-up of the host and the social conditions of the host population. We shall consider the genes that predispose to mycobacterial infection later. Notice also, the evidence that macrophages have co-evolved alongside mycobacteria and that special mechanisms have evolved for handling glycolipid antigens of mycobacteria and that macrophages are activated by components of the mycobacterial cell wall.

(Some of the material in the lecture concerned with antigen presentation anticipates material in the following section of the book. At this stage do not be concerned if some of the terms are unfamiliar, since this will be covered below, and you can always run through the lecture again after you have covered the additional areas.)

After listening to the mini-lecture and with your knowledge of macrophage functions from Section 2.5, you should be able to answer the following questions (the answers are given at the end of the book).

Question 2.7

What evidence is there that macrophages and TH1 cells are important in immunity to tuberculosis?

Question 2.8

What macrophage killing mechanisms are active against mycobacteria?

Question 2.9

What cytokines are important in the response to tuberculosis?

Question 2.10

How can we determine whether an individual has been exposed to tuberculosis?

Question 2.11

What factors determine whether an individual who has been infected with the tuberculosis bacteria will develop the disease?

2.6.3 Leprosy

While considering mycobacteria, we should also mention leprosy, since it illustrates a particular characteristic of macrophage-mediated immunity. *Mycobacterium leprae*, the leprosy bacteria, are usually transmitted by nasal secretions and, as they are very slow-growing, the incubation period is up to ten years. The bacteria typically grow in the skin, but in some cases affect other organs including nerves, and they may survive in macrophages in some individuals. The unique characteristic of leprosy is that the type of immune response that develops in different individuals varies enormously (Figure 2.21). Some patients develop a very strong cell-mediated immune response, which eliminates virtually all of the bacteria. Indeed the response can be so powerful that it causes a lot of damage in the host.

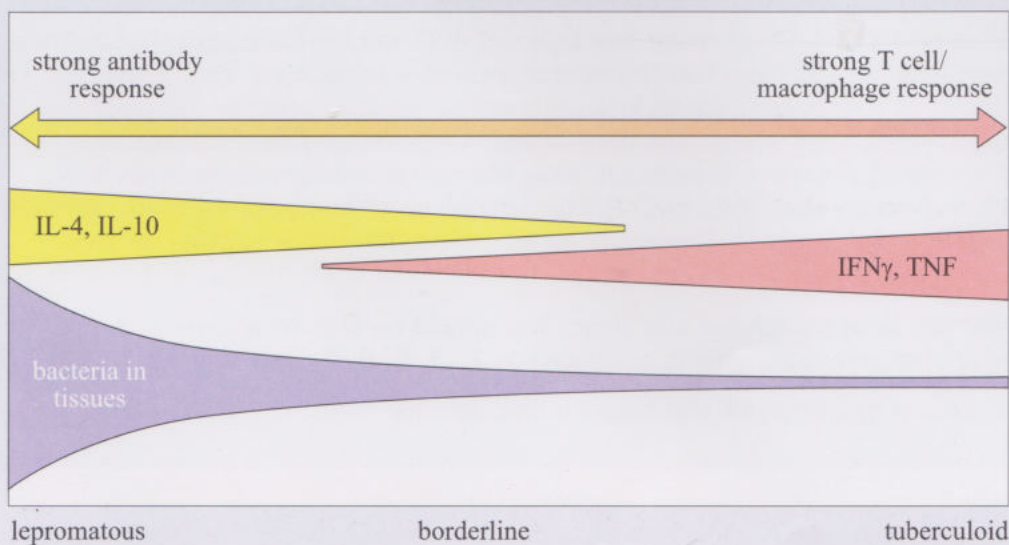


FIGURE 2.21

In lepromatous leprosy there are many bacteria in the tissue, despite the strong antibody response. In tuberculoid leprosy there are few bacteria and a strong cell-mediated response. The profile of cytokines found in the tissues reflects the prevalent type of response. The photograph shows an individual with a borderline reaction. He has many bacteria in the skin lesions and the developing response produces clearly defined areas of inflammation with cellular infiltration.

Such patients are said to have a 'tuberculoid' type of immune response. In contrast, other individuals produce no cell-mediated responses at all, although they do produce a good antibody response against the bacteria. These patients are said to have a 'lepromatous' type of response. Unfortunately, the antibody response is not effective against leprosy and the skin and other tissues of these patients is teeming with millions of bacteria. In these cases, it is the bacteria that produce damage throughout the body.

- ☐ What types of immune responses do tuberculoid and lepromatous leprosy represent?
- ☒ Tuberculoid leprosy reflects a T_H1 -type immune response, while lepromatous leprosy reflects the activation of B cells and antibody production directed by T_H2 cells, i.e. it is a T_H2 -type response.

Interestingly, patients can swing between having one type of immune response and another; as they do so the external appearance of the lesions on the skin changes (Figure 2.21). The problem with leprosy is that the bacteria are extremely resistant to destruction by macrophages. Once it has gained a hold in the individual, neither a strong cell-mediated response (T_H1) nor a strong antibody response (T_H2) is sufficient to eliminate it. In this disease, drug therapy is the best option.

In summary, phagocytes are clearly essential for immune defence against many bacteria and parasites. Several bacteria and parasites, e.g. *Leishmania*, which produce chronic infections, infect and can survive in macrophages. Macrophage activation mediated by T_H1 cells and $IFN\gamma$ is essential in stimulating the cells to destroy such invaders at an early stage, or else a chronic infection may develop.

2.7 Overview of antibacterial defences



The preceeding sections have looked at the ways in which the immune response deals with bacterial infections, and particularly the roles of antibodies, complement and macrophages. Clearly, the T cells are also important, but at this stage we shall take an overview of the mechanisms available to protect us against bacterial infections. We shall also consider some of the ways in which bacteria aim to evade immune defences.

You should now work through the topic on CD1 entitled *Antibacterial defences*, which is accessed from the Navihedron icon of a bacterium. You should use this session as an opportunity to look up anything on antibacterial defences and on phagocytosis that you do not understand, by accessing the screens of additional information. In addition, view the two expansion screens on how bacteria evade host defences 'Bacterial evasion of complement' and 'Bacterial evasion of phagocytes'. These act as an introduction to a section on this subject in Chapter 5.

After you have completed the topic, you should be able to answer the associated self-assessment questions 1, 2, 3, 5, 6, 7, 8, 9 and 10.

You should spend about one hour on this activity.

2.8 Eosinophil reactions to helminths

Eosinophils were introduced briefly at the beginning of the course, in order to complete the roll call of the different types of granulocyte (Figure 1.6). We also noted there that eosinophils have an important function in defence against parasitic worm infections. This section expands on the functions of eosinophils, which use a quite different type of immune defence to macrophages. After studying this section, you will be able to contrast macrophage-mediated defences that are primarily controlled by Th1 cells with eosinophil-mediated reactions that are promoted by Th2 cells.

Eosinophils are present in small numbers in the blood; they constitute 2–5% of the blood leukocytes in a normal individual, although the number can rise significantly in individuals infected with parasitic worms and in some people who have allergies, for example, severe hayfever, and asthma. At sites of worm infection in the gut or lung eosinophils may constitute the main leukocyte population.

- How is it possible to have low numbers of eosinophils in the circulation and high numbers at the site of worm infection?
- Eosinophils are selectively attracted to the site, because they respond to chemotactic molecules released from that area, whereas other cells, such as neutrophils, respond less well to those signals because they lack the necessary receptors.

Eosinophils have the irregular nucleus characteristic of granulocytes and they contain numerous granules with a central crystalloid core, which consists largely of a toxic protein called major basic protein (Figure 2.22). (Do not confuse major basic protein (MBP) with myelin basic protein, also called MBP, which is a

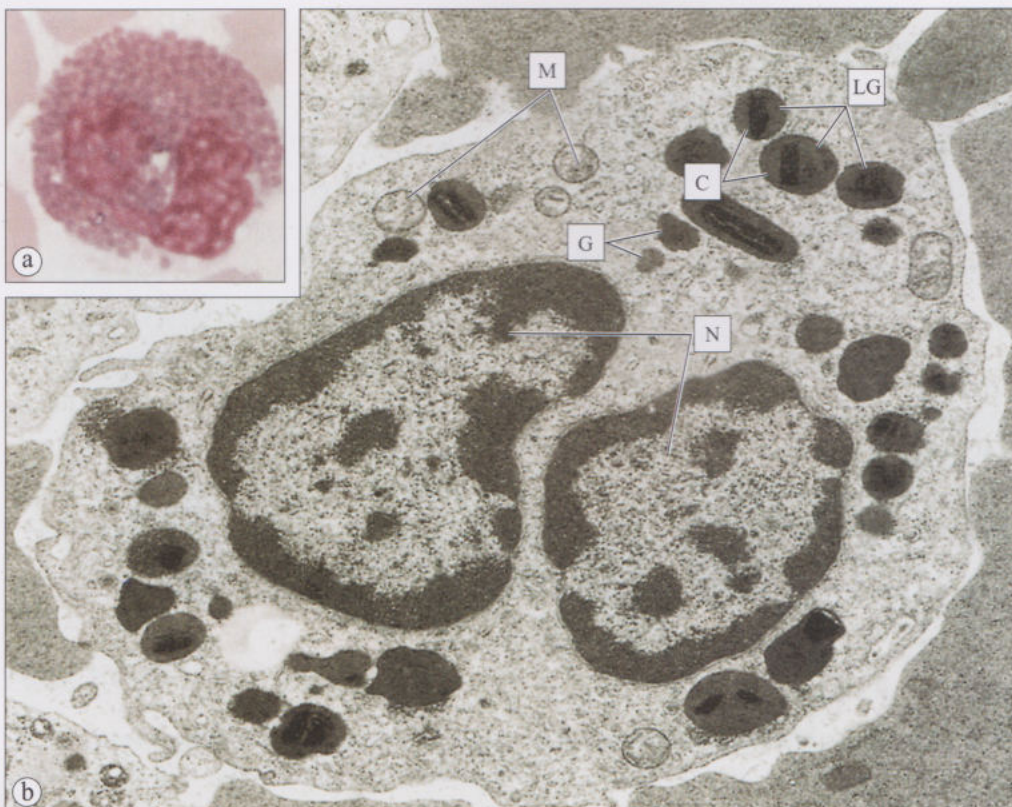


FIGURE 2.22

(a) An eosinophil viewed under the light microscope. (b) The ultrastructure of an eosinophil seen in a transmission electron micrograph. The cell contains a bilobed nucleus (N), mitochondria (M) and characteristic large alkaline granules (LG) containing major basic protein in the crystalloid core (C). Smaller granules (G) contain enzymes involved in modulating inflammation.

component of cells in the nervous system.) Other highly toxic cationic molecules are found in their granules, including one that is very effective at killing the schistosomes of trematodes such as *S. mansoni*, that lives in the venous circulation and the liver. The granules also contain a peroxidase, that can generate reactive oxygen intermediates, in a process analogous to that used by neutrophils and macrophages. Here the similarity ends; eosinophils do not generally phagocytose their targets.

- Why is phagocytosis not an appropriate way for an eosinophil to attack a worm?
- A parasitic worm is hundreds of times larger than an eosinophil. If you were asked to demolish a small building, you would not do so by trying to eat it. You would probably enlist the assistance of a few friends and set about it with sledge-hammers. In effect that is how eosinophils tackle parasites which are much larger than themselves.

Eosinophils attack their targets by recognising them, binding to them and then directing their granule contents to the outside – a process called exocytosis. In Figure 2.23 you can see eosinophils directing an attack on a schistosome. They have penetrated beneath the outer tegument of the worm. The granules fuse with the plasma membrane of the eosinophil, releasing the granule contents directly against the surface of the parasite. The worm is thus subjected to a very high concentration of toxic molecules.

The mechanisms by which eosinophils reach their target and then recognize it are conceptually similar to the ways in which neutrophils migrate towards and recognize their targets. Eosinophils have receptors that allow them to respond to a particular set of chemokines, including the 'eotaxins', which are produced by a number of different cell types, at sites of infection. The cells that produce eotaxin

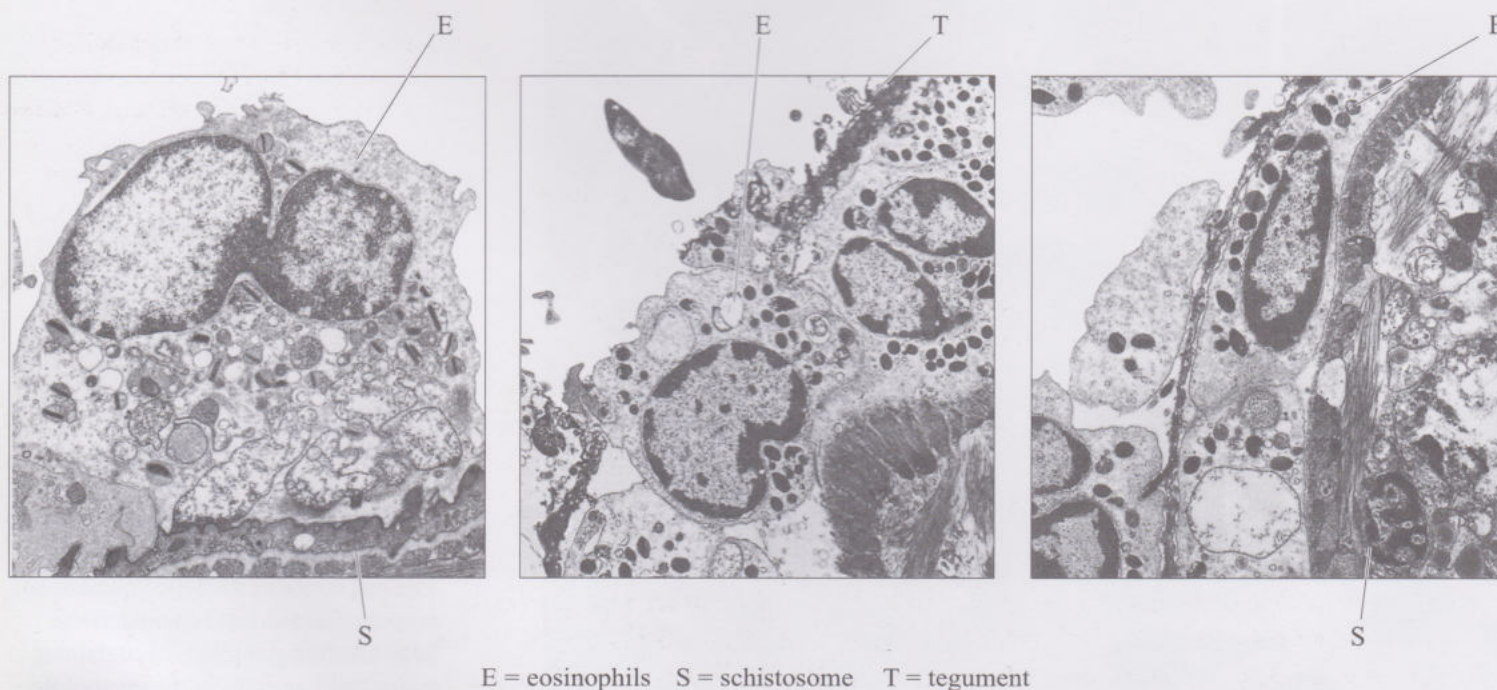


FIGURE 2.23 Eosinophils collectively can kill schistosomes. In this series of electron micrographs the eosinophil (E) adheres to the surface of the schistosome (S; left), then migrates through the outer tegument of the worm (T) and releases its granule contents, disrupting the worm surface (centre and right).

include endothelium, mast cells, epithelium in the airways, fibroblasts and keratinocytes in the skin. In addition to attracting eosinophils to sites of infection and inflammation, eotaxins prime them to produce their reactive oxygen intermediates. It is worth noting here that Th2 cells and macrophages also have the receptors which allow them to respond to eotaxins. This means that, when eotaxins are released at a site of infection, they will attract a specific set of cells that is appropriate to deal with one type of infection. This subject is still very much under investigation, because the ways in which cells respond to chemokines is complex. Nevertheless, you can see the principle that an appropriate set of cells is attracted to each type of infection by the local release of a particular set of chemokines and other inflammatory mediators.

Once eosinophils have reached the area, they can recognize the target, because of antibodies bound to its surface – opsonization. Eosinophils have receptors for IgG (FcγRIII and FcγRII), IgA and IgE (FcεRI), which allow them to bind to pathogens sensitized with these antibodies. Interestingly, IgE is also often increased in worm infections and high levels of IgE, and elevated numbers of eosinophils are characteristics of Th2-type immune responses (Figure 2.24).

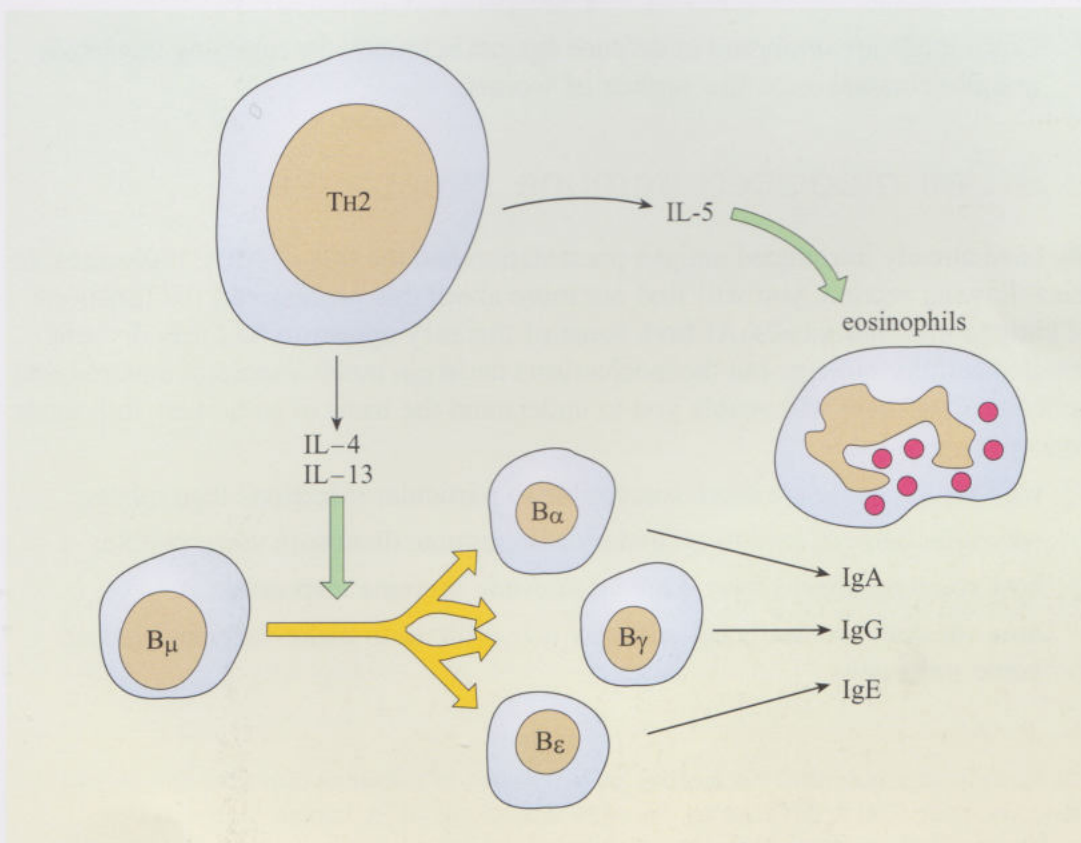


FIGURE 2.24

Th2 responses are characterized by the production of high levels of antibodies. Th2 cells and the cytokines they produce promote the differentiation of IgM-producing B cells (Bμ) into cells producing IgA, IgG and notably IgE (Bα, Bγ, Bε). Interleukin-5 promotes the development and differentiation of eosinophils, which together with specific antibodies are important in controlling worm infections.

Before moving on, we should look at one more strand of the Th2-type of immune response.

Th2 cells produce a cytokine, IL-5, which mobilizes eosinophils from bone marrow causing an increase in the numbers in the blood. In this function, IL-5 acts in concert with eotaxin that also promotes eosinophil mobilization. So, when there is a demand for eosinophils created by an infection and a Th2-type response, the bone marrow supplies additional cells of the appropriate type.

We have now identified another type of immune response, one that involves T_H2 cells, antibodies, and eosinophils. Later on we shall look at how T_H2 cells promote particular types of antibody response, and how the balance between T_H1 -type responses and T_H2 -type responses develops. However, by this stage you will have started to realize that the production of an appropriate immune response for a particular pathogen is determined by the T cells which are controlling the response, and the blend of cytokines and chemokines which they release.

Summary of Sections 2.5–2.8

- 1 Phagocytes are important in defence against many bacterial infections. They phagocytose pathogens and subject them to a barrage of toxic reactive oxygen and nitrogen compounds. Killed bacteria are digested by lysosomal enzymes.
- 2 Degraded antigens can be presented by macrophages to T cells.
- 3 Macrophages release IL-12 that promotes T_H1 cell production, while T_H1 cells release $IFN\gamma$ which activates macrophages.
- 4 Macrophages are particularly important in defence against intracellular bacteria, including typhoid, tuberculosis and leprosy.
- 5 Eosinophils are important in defence against helminths by releasing their toxic granule contents onto the surface of worms.

2.9 Cell-mediated immune responses

We have already introduced antigen presentation and the role of MHC molecules. In the following section, you will find out more about this process and the functions of antigen-presenting cells. At first, some of this may appear to be fairly dry and detailed cellular biology, but the mechanisms underpin much important material and understanding them will enable you to understand the basis of some very important concepts, such as:

- why some people are more susceptible to particular infections than others;
- why infection can lead to secondary autoimmune disease in some people;
- how some pathogens have evolved to evade immune responses;
- how vaccines are designed, and why it is difficult to make vaccines against some pathogens.

In this section we shall be looking at a number of complex dynamic processes, and you may find it difficult to visualize exactly what is happening from the diagrams and written description alone. If you do find it hard to understand, I suggest you take a quick preview of the CD1 animation entitled *Antigen processing and presentation* (accessed via the Navihedron icon of a macrophage presenting antigen to a T cell) that comes at the end of Section 2.9. When you have seen the animation, finish reading through the text and return to work through the CD-ROM material thoroughly at the end of the section.

You are not expected to memorize the molecular structures shown in this section, but looking at them will help you to understand their functions.

2.9.1 MHC molecules and antigen presentation

First we must distinguish two different pathways of antigen presentation. The pathway by which all nucleated cells sample their internal molecules and present them on the cell surface is called the **class I pathway** or **internal pathway**, because the cell is taking its own intrinsic, internal molecules and placing them on its cell surface. To do this it uses 'class I molecules' encoded within the major histocompatibility complex (see below). The presented molecules are reviewed (i.e. potentially recognized) by cytotoxic T cells (Figure 2.25).

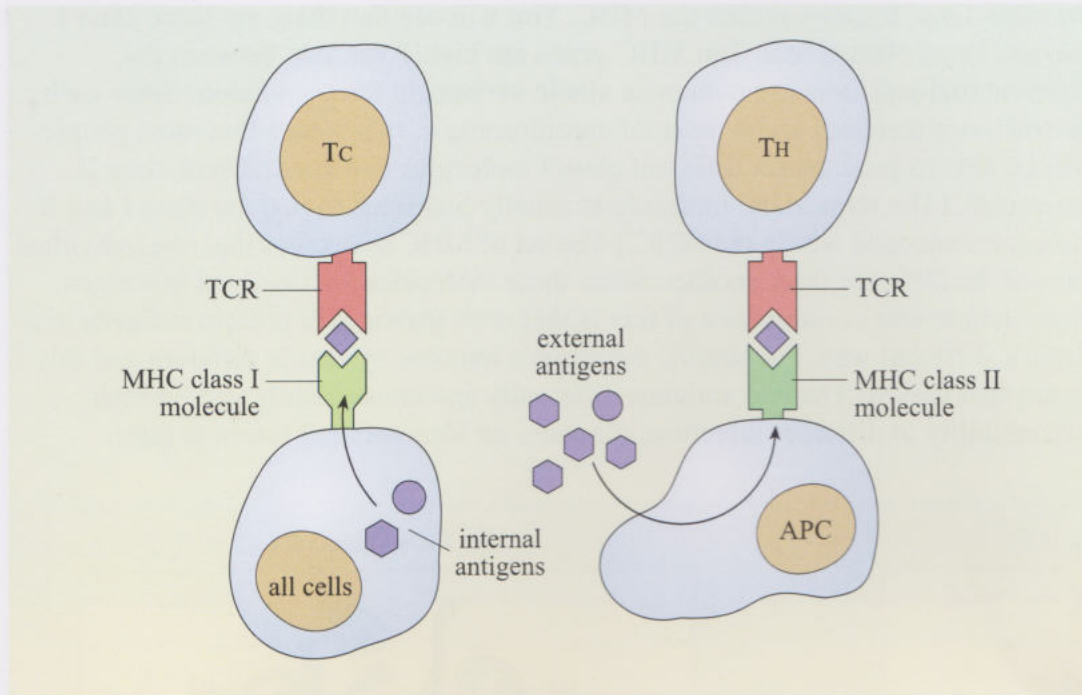


FIGURE 2.25

All cells of the body process their internal molecules (antigens) and present peptide fragments on the cell surface associated with MHC class I molecules for review by cytotoxic T cells. Antigen-presenting cells (APCs) such as macrophages internalize molecules and process them into peptide fragments that associate with MHC class II molecules, which are recognized by helper T cells.

The second pathway is the one you have already seen used by the macrophage. In this the cell takes up molecules from the outside by phagocytosis and presents them on the cell surface associated with MHC 'class II molecules'. Because the presented molecules come from outside the cell, this is called the **external pathway** or **class II pathway**. The presented molecules are reviewed by helper T cells (Figure 2.25).

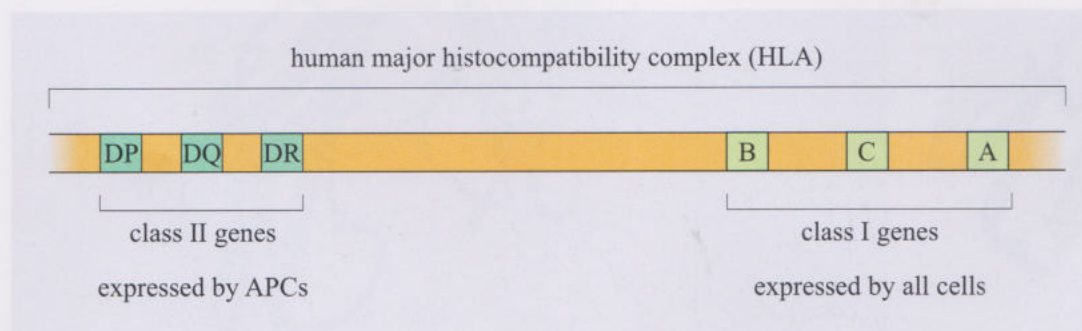


FIGURE 2.26 The major histocompatibility complex in humans was first identified because MHC molecules could be detected (using antibodies) as antigens expressed on leukocytes, so it was called the human leukocyte antigen locus or HLA. There are three class II loci, HLA-DP, -DQ and -DR and three class I loci HLA-A, -B and -C. The genes are codominantly expressed, so for example, a cell will produce six different types of class I molecule, -HLA-A, -B and -C – three from the maternal chromosome and three from the paternal chromosome.

The major histocompatibility complex

The terms 'MHC class I' and 'class II molecule' need some explanation, and this involves a short excursion into the major histocompatibility complex. The **MHC** is a large group of linked genes, located on chromosome 6 in humans. The gene complex was originally identified because of its role in determining whether tissue would or would not be rejected, when grafted between individuals, but clearly this is not a physiological role. There are at least 150 genes in this region with a variety of functions, but we shall concentrate initially on the so-called class I and class II genes that are involved in antigen presentation. Figure 2.26 shows the location of the class I and II genes within the MHC. You will see that there are three class I loci and three class II loci. The MHC genes are highly variable between the different loci and there are numerous allelic variants in the population. Since each person has a maternal and a paternal chromosome 6, this means that most people will be able to produce six different class I molecules and six different class II molecules. (The term MHC molecule is usually restricted to just the class I and II molecules encoded within the MHC.) The set of MHC molecules that one individual has will be different from another. Since these molecules are involved in antigen presentation, one consequence of this is that each person will present antigens in a slightly different way. Put simply, everyone's immune system is different and this means that they will handle antigens differently and consequently vary in their susceptibility to different infectious diseases, an idea we shall return to later.

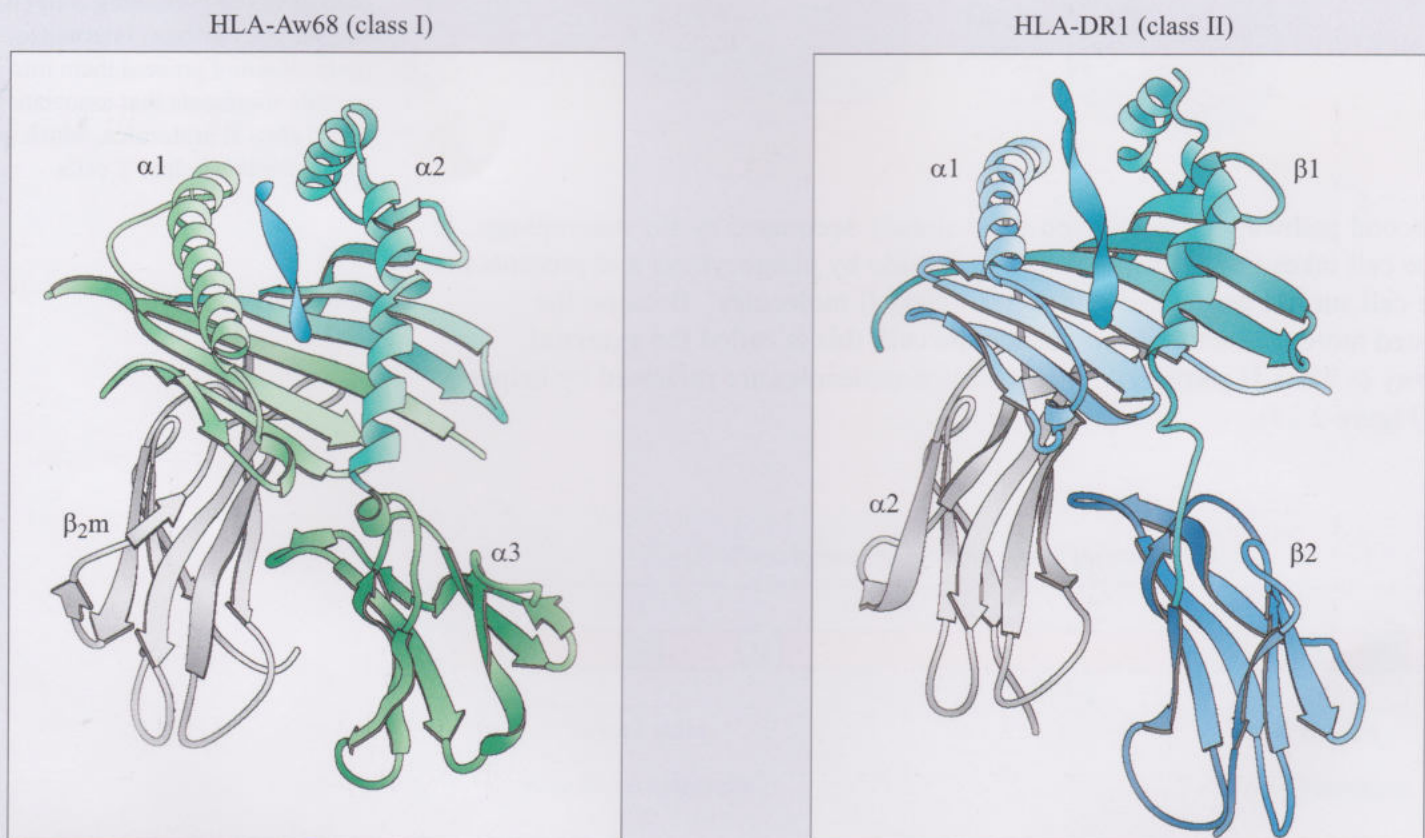


FIGURE 2.27 MHC class I (left) and class II (right) molecules shown as ribbon diagrams of the extracellular portions. The class I molecule has one chain encoded within the MHC which folds into three domains $\alpha 1$, $\alpha 2$ and $\alpha 3$ and a second smaller chain $\beta 2$ -microglobulin (β_{2m}) which is encoded on another chromosome. The antigen-binding site is formed by two loops of α -helix supported on a grid of β -pleated sheet and it is occupied by a peptide of eight amino acid residues. The class II molecule has two MHC-encoded peptides, but the overall structure is clearly very similar. The binding site can accommodate larger peptides.

MHC molecules

The class I and II molecules are structurally similar integral membrane proteins (Figure 2.27). They have two domains lying next to the cell membrane, and the distal part of the molecule consists of a grid of β -pleated sheet, surmounted by two long loops of α -helix, forming a cleft – the antigen-binding cleft. In class I molecules, the cleft is closed at each end and can accommodate polypeptides of eight amino acid residues in length. In class II molecules, the cleft is open at the ends and can accommodate polypeptides of 12–14 residues.

When an MHC molecule presents an antigen to a T cell, it actually presents a peptide fragment of the antigen. The receptor on the T cell recognizes not just the antigenic peptide but the combination of a specific peptide associated with a particular MHC molecule. In practice this means that the amino acid residues which form the T cell antigen receptor interact with both residues of the antigenic peptide and residues on the α -helices of the MHC molecule. The 'view' which the T cell receptor sees as it approaches the MHC/antigen complex is shown in Figure 2.28. You can see the antigenic peptide nestling in the groove at the top of the molecule.

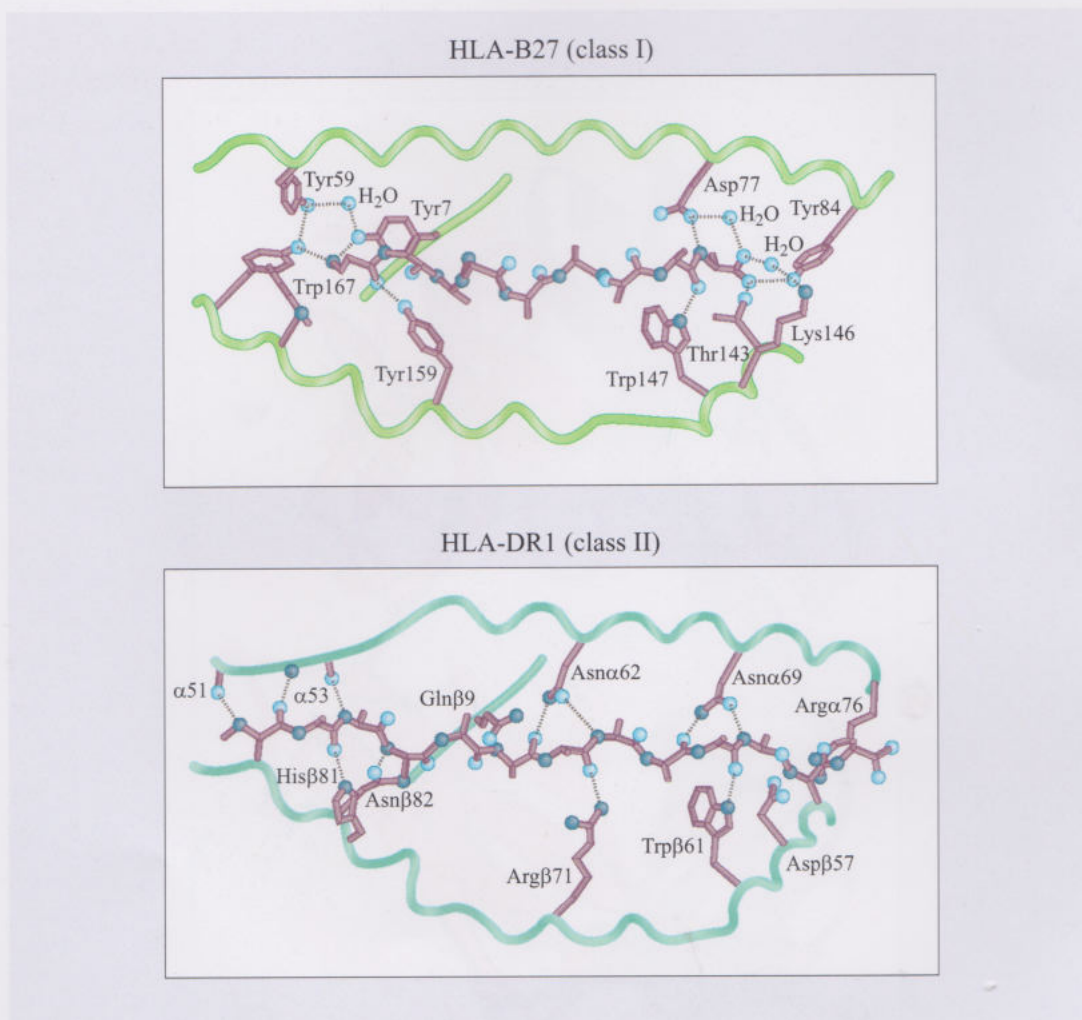


FIGURE 2.28 The peptide binding site of an MHC class I molecule (HLA-B27) and a class II molecule (HLA-DR1) is shown, as 'seen' by the T cell receptor. Antigenic peptides are shown binding in the groove. The peptide in the class I groove is shorter and is anchored by non-covalent hydrogen bonds at each end. The peptide in the class II groove is longer and is anchored to residues on the MHC molecule along its length. The labels indicate the amino acids in 3-letter code, and their position in the polypeptide chains.

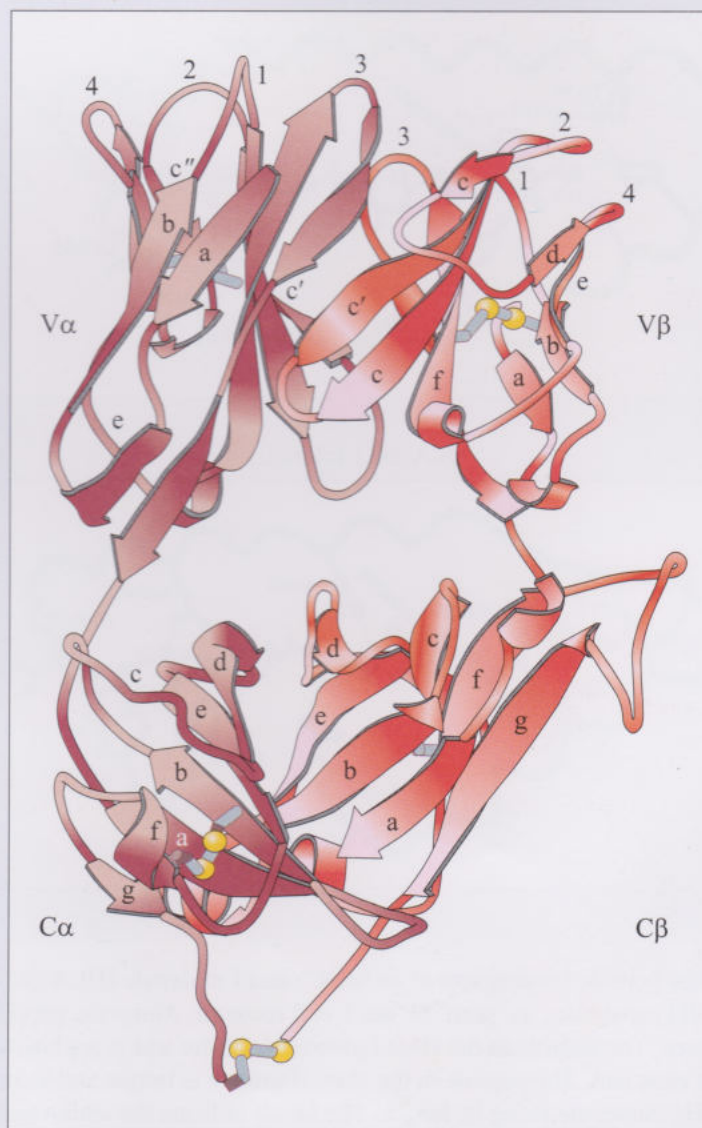
- ☐ Can you see any advantage in having a variety of different MHC molecules, each with a slightly different structure, but each capable of presenting antigenic peptides?
- ☒ The peptides which can bind to an MHC molecule depend on the particular allelic variant. If a person has a variety of MHC molecules, their cells can present a wider variety of antigens. This means that their immune system can respond efficiently to a wider variety of pathogens.

The T cell receptor

Although we only described the T cell receptor (TCR) very briefly in Figure 1.7, it has many similarities to the Fab region of an antibody molecule (see Figure 2.1). It is formed from two polypeptide chains, each of which has two domains, with the outer domains being highly variable between different T cells. Each of the variable domains has three hypervariable loops, so that the binding site for the MHC/antigen complex is principally formed from six hypervariable loops. You can see that this is very similar to a single antigen-combining site on an antibody (Figure 2.29). Also, although we did

FIGURE 2.29

The T cell receptor is formed from two polypeptide chains, alpha and beta, each of which has a constant domain ($C\alpha$ and $C\beta$ respectively) and a variable domain ($V\alpha$ and $V\beta$). The folding of the polypeptide chains in the V domains causes a cluster of hypervariable loops (numbered) to come together at the distal tip of the molecule. This region forms the binding site for the MHC/antigen peptide complex. The binding site is principally formed by the six hypervariable loops (1–3) clustered at the top of the molecule, although additional polypeptide loops (4) can also contribute. The letters 'a–g' indicate the successive strands of β -pleated sheet making up each of the domains. The domain structure is similar to that present in antibodies.



not go into the mechanisms in detail, the way in which T cells generate their repertoire of different TCRs is very similar to the way in which B cells generate their repertoire of different antibodies. Of course, like the B cells, the T cell receptors are clonally distributed, meaning that each T cell only has one type of receptor, even though the population of T cells as a whole has an enormous range of receptors.

Antigen processing

We now look at how the antigenic polypeptides come to be associated with MHC molecules, a process called **antigen processing**. We will consider first how molecules are processed for the class I pathway. All nucleated cells express MHC class I molecules and present antigens via this pathway.

- ☐ Why is it advantageous for all nucleated cells to present antigen via the class I pathway? Put another way, why might any cell of the body need to present antigen to T cells?
- ☒ Any nucleated cell of the body may become infected with a virus. In order to be surveyed by Tc cells every cell needs to present antigen. The MHC class I molecules carry out this function, so every cell has them.

Within cells there is an organelle called the proteasome whose function is to take protein molecules that the cell has tagged for breakdown and chop them up into polypeptide fragments. All cells turn over and renew their proteins, so this is a perfectly normal cellular function. Transporter molecules now take some of these fragments and carry them into the endoplasmic reticulum, where MHC class I molecules are synthesized. The peptides are loaded into the cleft on the MHC molecule where they bind specifically, but non-covalently to amino acid residues around the cleft. Like antigen-antibody binding, the antigenic peptide and the MHC molecule form multiple non-covalent bonds, and the shape of the peptide is complementary to the shape of the antigen-binding cleft. The MHC class I molecules with their bound antigenic peptides are now transported to the cell surface (Figure 2.30).

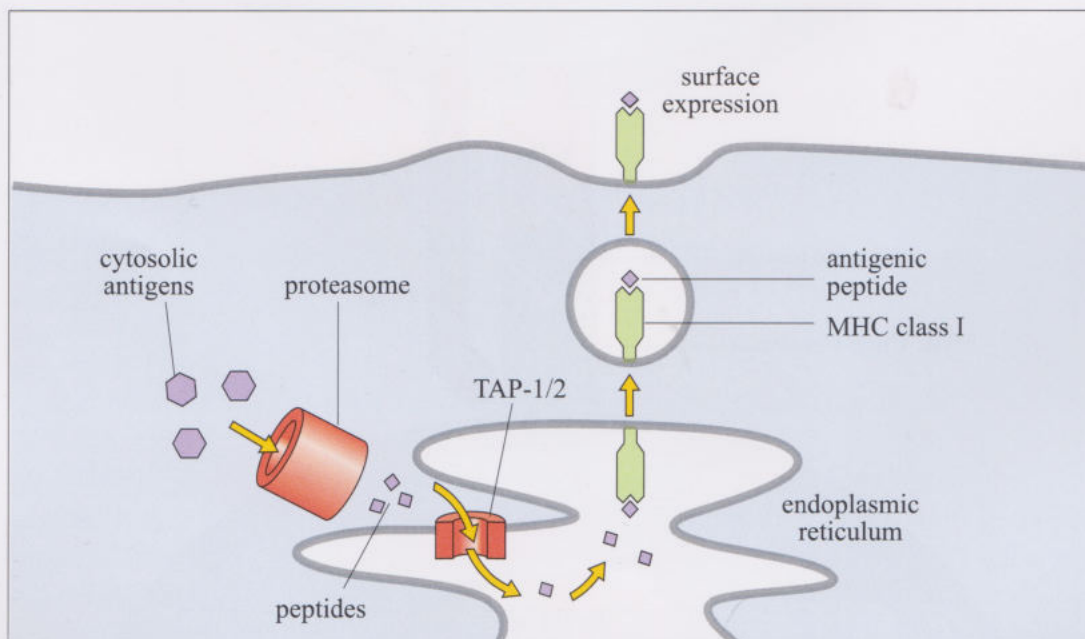


FIGURE 2.30

Cells degrade cytosolic antigens in an organelle called the proteasome. The fragments are transported to the endoplasmic reticulum by a specialized transporter (TAP1/2), and become associated with MHC class I molecules which have been synthesized in the endoplasmic reticulum. The MHC/peptide complex is moved to the cell surface for review by cytotoxic T cells.

Once they have arrived at the cell surface, the antigen-loaded MHC molecules can interact with the T cell receptor on Tc cells. Once again the interaction is non-covalent. A tri-molecular complex is formed in which the antigenic peptide occupies the groove on the MHC molecule and the T cell receptor interacts with residues on both the antigen and the MHC molecule.

- If the gene for an antigen mutated causing a change in an antigenic peptide, do you think that the change in the peptide would affect its ability to form the trimolecular complex of MHC molecule/antigen/TCR? If so, what would be the effect, and how would it be mediated at a molecular level?
- Changes in the antigenic peptide often, but not always, affect the formation of the trimolecular complex. Mutation of residues involved in the binding to the MHC molecule may prevent the antigen from binding to the groove on that particular MHC molecule. Likewise, mutation of residues sticking out of the groove, can prevent the TCR from binding to the MHC/antigen complex. Put simply, if an antigenic peptide has mutated it is likely that a T cell that recognized the original peptide cannot now do so or will do so less efficiently.

The function of CD8

The CD8 molecule (a characteristic marker of Tc cells) is also involved in the interaction between the T cell and a cell expressing the class I molecules. It binds to a site on the MHC class I molecule, in one of the domains adjoining the membrane, that is, distant from the antigen-binding cleft (Figure 2.31). We now have four molecules interacting at the cell surface. CD8 is important in initiating the intracellular signals that activate the T cell. It does this by bringing an enzyme into proximity with the T cell receptor that phosphorylates CD3 (which you may remember is part of the T cell receptor). A complex series of interactions follows that leads to the activation of the T cell.

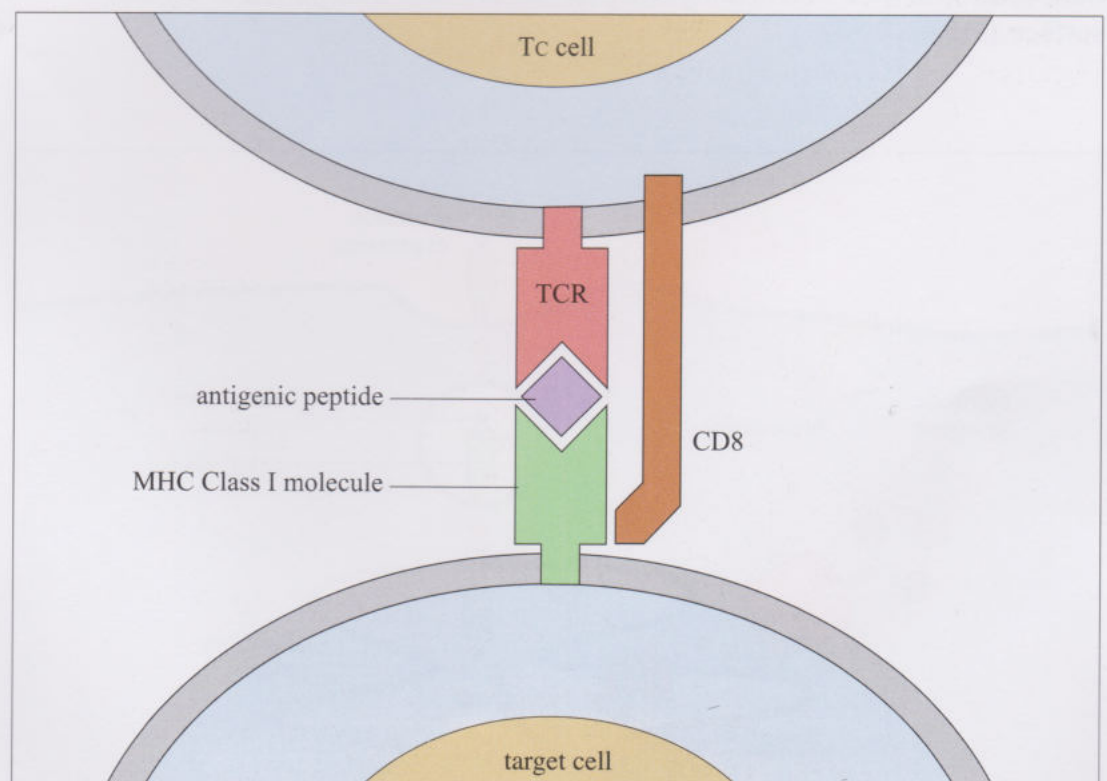


FIGURE 2.31
T cell recognition of antigen involves the interaction of four molecules. The antigenic peptide bound to the MHC molecule on the target cell is recognized by the T cell receptor (TCR) which interacts with residues on both the peptide and the MHC molecule. In the class I pathway the CD8 molecule on the cytotoxic T cell binds to an invariant part of the MHC class I molecule.

If a cytotoxic T cell recognizes the antigen presented by the MHC molecule as foreign then it is able to kill the target cell. The way in which it does this is rather subtle. In fact, it instructs the target cell to commit suicide. We shall return to examine how this takes place, when we look at anti-viral defences, but we will continue here by looking at the class II pathway, comparing it with the class I pathway.

The class II pathway

Antigen presentation via the class II pathway is carried out by dendritic cells, mononuclear phagocytes and B cells, the principal cells in the body which express MHC class II molecules. However in some circumstances, particularly at sites of inflammation, and in response to $\text{IFN}\gamma$, cells from the tissues (not leukocytes) may also be induced to express class II molecules. However such tissue cells are often less effective at presenting antigen, because they lack costimulatory molecules. So we shall consider a good antigen-presenting cell, the macrophage.

As you saw earlier (Figures 2.19 and 2.25), macrophages that have degraded microbial antigens in their phagosomes can return the antigenic peptides to the cell surface for presentation to T helper cells. The MHC class II molecules are synthesized, like the class I molecules on the endoplasmic reticulum. From here, vesicles bud off, carrying the class II molecules to an acidic endosome (vesicle) compartment within the cell called the MIIC compartment. Vesicles containing degraded antigenic peptides from the phagosome fuse with the MIIC compartment, so that the MHC molecules and the peptides meet at this point. Within the MIIC compartment the antigenic peptides are loaded onto the class II molecules. Class II molecules are synthesized with a polypeptide occupying the antigen-binding cleft, and this is displaced and degraded as the antigenic polypeptides are loaded. The process of loading both class I and class II molecules with antigen is complex and involves a number of other molecules, but this goes beyond the scope of the course. The peptides that have been loaded onto the class II molecules are trimmed to size, by proteases. Then the MHC molecule, loaded with antigenic peptides, moves towards the cell surface to be expressed on the plasma membrane. The process is illustrated diagrammatically in Figure 2.32.

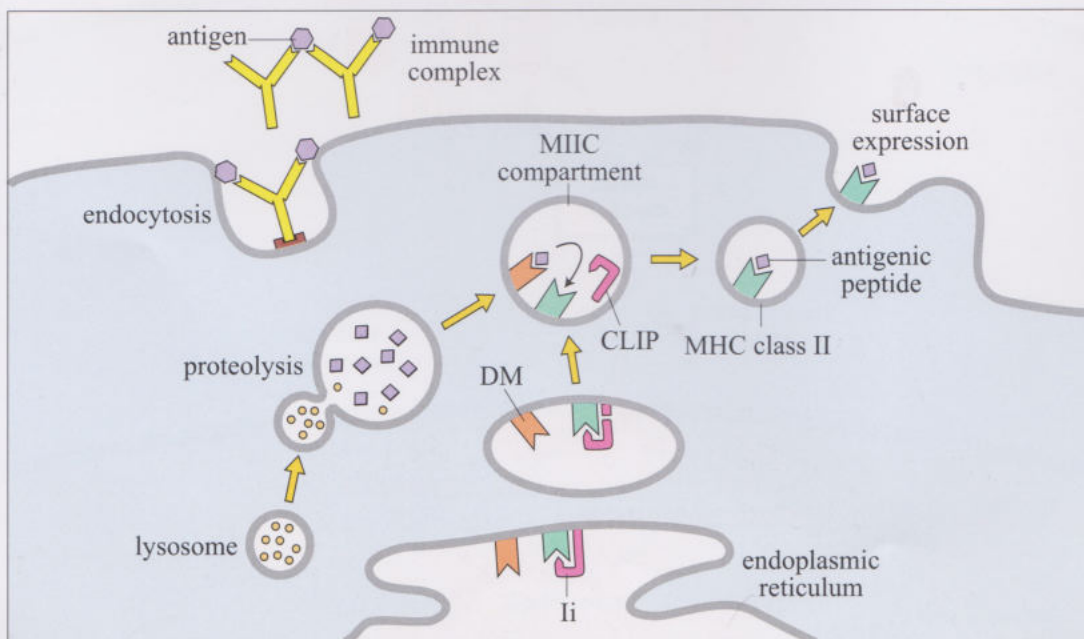


FIGURE 2.32

Antigen can be taken up by antigen-presenting cells in several ways, such as that shown here in antigen-antibody immune complexes. The endocytosed material is broken down into fragments by proteolysis. Meanwhile MHC class II molecules are synthesized in the endoplasmic reticulum with a chain (Ii) blocking their combining site. The peptide fragments and MHC molecules meet each other in the MIIC compartment and fragments are loaded onto the MHC molecule with the aid of another MHC-encoded molecule called DM. At this stage, the Ii chain is cleaved into a fragment called CLIP and replaced by the antigenic peptide. The MHC class II molecule loaded with antigenic peptide is now transported to the cell surface to present antigen to T helper cells. In practice, a single cell will express thousands of MHC molecules loaded with a variety of different peptides.

Once they have arrived on the plasma membrane, the MHC/antigen complex can be recognized by receptors on helper T cells. In this case the CD4 molecule, which is a characteristic marker of the T helper cell, binds to part of the MHC class II molecule in an analogous way to the binding of CD8 to MHC class I (compare Figures 2.19 and 2.31). Indeed, you can see that the way in which T_H cells and T_C cells recognize antigen is very similar, although the ensuing reactions are quite different.

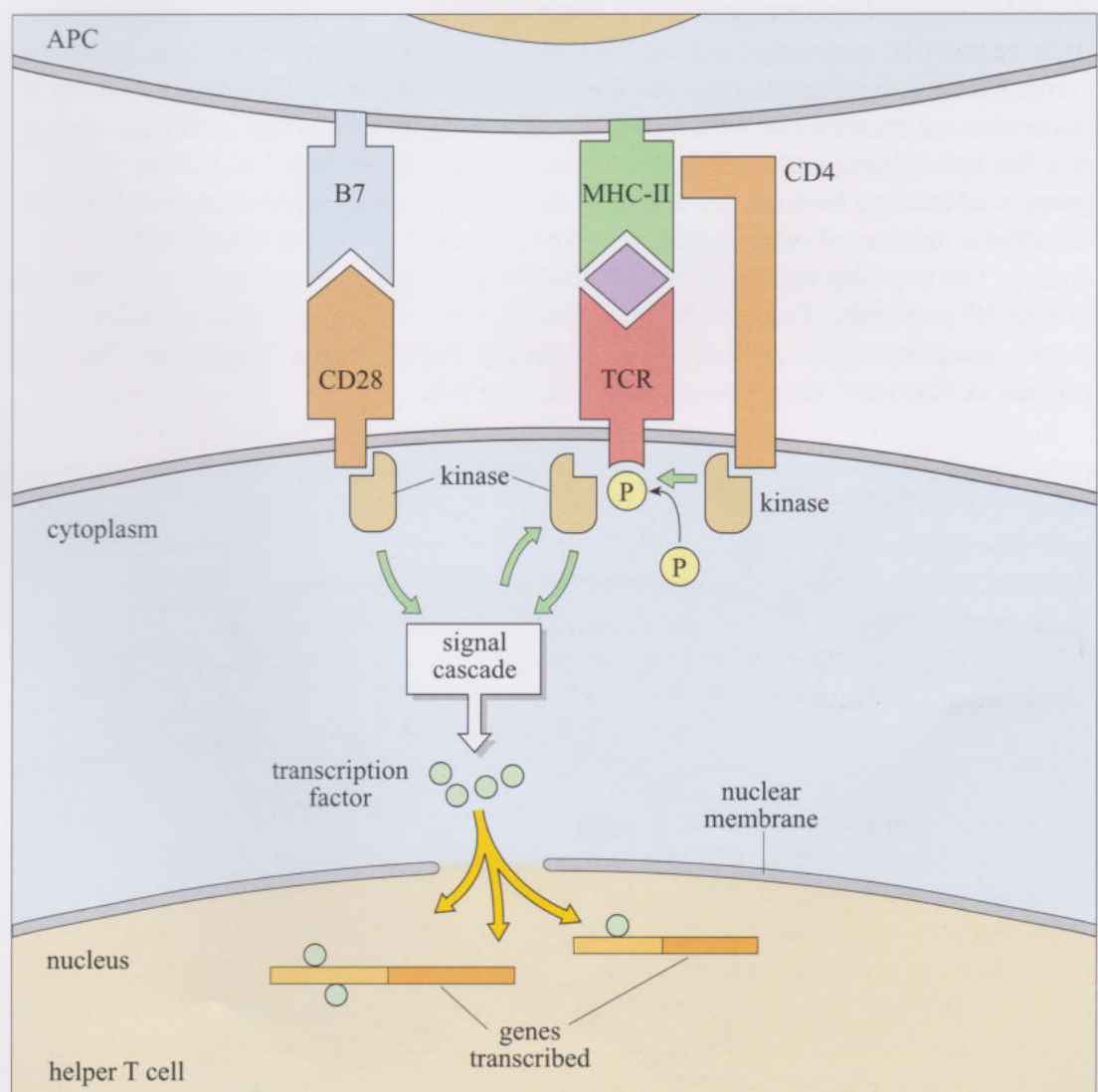
Costimulation

Although recognition of an antigenic peptide is the first requirement to activate a T_H cell, other interactions are also required before it will divide or release its cytokines. The most important additional interaction is 'costimulation'. Although what follows is simplified; if a T cell recognizes antigen/MHC and receives a costimulatory signal, then the cell will be activated; if the cell sees antigen but does *not* get a costimulatory signal it will not be activated.

Important costimulatory molecules which are expressed on antigen-presenting cells are B7-1 and B7-2 (CD80 and CD86 respectively), which interact with CD28 present on the T helper cell (Figure 2.33). The costimulatory molecules are typically expressed on mononuclear phagocytes, and are increased by stimulation with microbial components, such as lipopolysaccharide or by cytokines which are

The term 'ligand' is a general description of any molecule that binds non-covalently to another, e.g. CD28 to B7. 'Ligation' refers to the binding.

FIGURE 2.33
Helper T cells are activated by a dual signal from the antigen receptor and the costimulatory molecule CD28. When the T cell receptor binds antigen the CD4 molecule is brought into proximity with the receptor. A kinase enzyme phosphorylates the T cell receptor which now recruits another kinase to the receptor complex. Ligation of CD28 similarly activates yet another kinase. The two receptors (TCR and CD28) now initiate a signalling cascade which leads to the production of transcription factors which move into the nucleus and bind to the promoters on genes required for T cell activation, causing them to be transcribed.



produced in inflammation. The T cell integrates a signal from its antigen receptor (TCR) and CD28 within the cell. Intracellular signalling pathways intersect and interact with each other so that the cell is only activated if *both* signals are received. Although much is known about intracellular signalling in the immune system, the detailed cellular biology is not included in this course.

- ☐ Can you see any advantage in the T cell requiring two signals to become activated, i.e. correctly presented antigen and then costimulation?
- ☒ Antigen-presenting cells present antigen all the time and mostly the antigens are harmless molecules, so an immune response is not appropriate; it could even be damaging. However, if infection or inflammation occurs, an immune response is appropriate, and this is when the costimulatory molecules are most highly expressed. Having two signals is a failsafe device, so immune responses are only promoted when they are appropriate.

While this discussion has concentrated on the interactions which occur between Th1 cells and mononuclear phagocytes, an analogous set of costimulatory actions takes place when Th2 cells interact with cells, and we shall return to this later when we look at how T cells help B cells to make antibodies.

The role of adhesion molecules

In addition to the costimulatory molecules, a number of other cell surface molecules may be involved. For example the molecules LFA-1 and ICAM-1 which you encountered earlier on endothelium, may also be involved in the interactions between T cells and antigen-presenting cells. It is important that the T cells and the antigen-presenting cells stay bound to each other for long enough for the T cell to receive appropriate stimulation and costimulation. Several molecule pairs can contribute to this adhesion, and may enhance the effectiveness of the signalling between the two cells.

How many signals?

It is now worth considering how big a signal a T cell needs to receive, for it to become activated. The diagrams in Figures 2.31 and 2.33 show just one MHC molecule, and one T cell receptor. However a cell could have more than 100 000 MHC molecules present on its surface. Of these, perhaps only 100–200 are required to bind to receptors on the T cell, in order to activate it. In other words, a T cell only needs to recognize about 0.1% of the MHC/antigen complexes on the surface of a cell in order to respond to it. If you think about it, this seems appropriate, because the cell may express hundreds of different MHC/antigen molecules, from all the different antigens that it is processing. Therefore, only a tiny proportion of the total can be recognized by any one T cell.

T cell proliferation

The events that follow T cell activation depend on cytokine signals, and on where the interactions take place. In lymph nodes or other secondary lymphoid tissues, T cells generally go on to divide. An important cytokine for T cell proliferation is IL-2 (originally called T-cell growth factor). When a T cell is activated it synthesizes IL-2 receptors and expresses them on its surface and it also synthesizes IL-2. Thus it can respond to the cytokine it produces, or which other T cells in the vicinity are producing. This is an example of autocrine stimulation. With this final signal, the T cell undergoes a number of cycles of cell division (Figure 2.34).

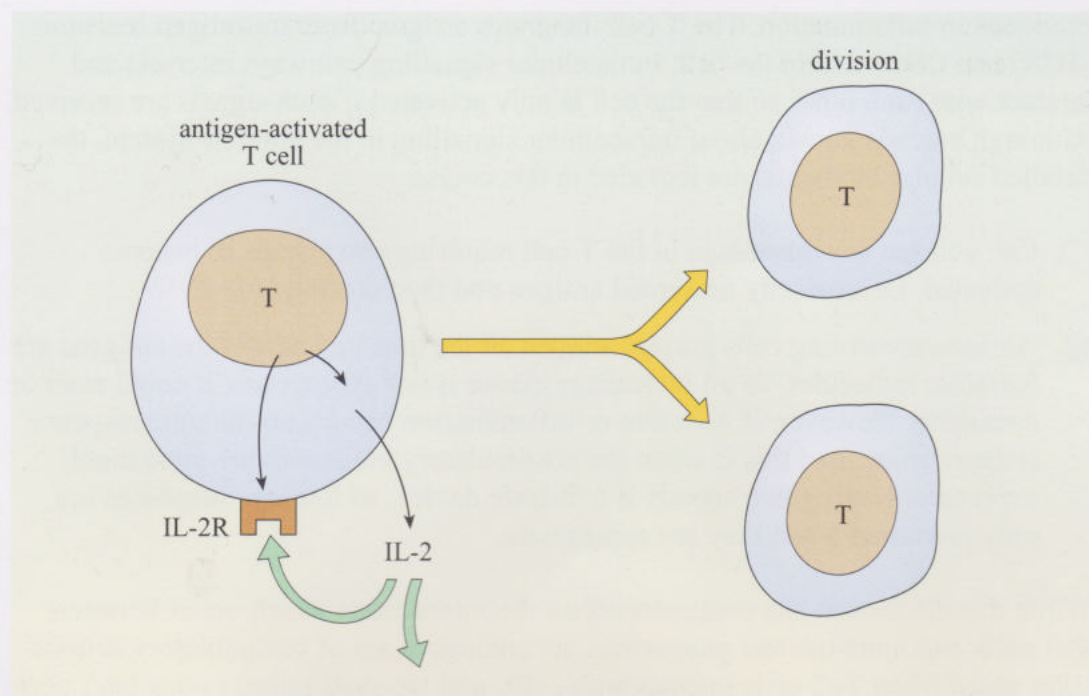


FIGURE 2.34

When T cells are activated they transcribe the genes for interleukin-2 (IL-2) and for its receptor (IL-2R). Secreted IL-2 can act on the cell that produced it and on other activated T cells in the vicinity. T cells that have been stimulated by IL-2 undergo several rounds of cell division.

Activation of the T cell therefore depends on the availability of IL-2 and whether the cell is expressing IL-2 receptors. The IL-2 receptor itself is not a simple molecule. It can exist in high and low affinity forms. At rest a T cell expresses just the low affinity form in small numbers, so it takes a high concentration of IL-2 to stimulate the cell to divide. When the cell has been activated by antigen, the cell synthesizes an extra chain for the receptor which converts it into a high affinity form. Many more of these high affinity IL-2 receptors are produced, so that the cell will respond much more readily to low amounts of IL-2 (Figure 2.35). This means that the response of a T cell will depend, not just on the levels of IL-2 present, but also on the numbers and type of IL-2 receptors. The expression of the IL-2 receptors can be enhanced by other factors, such as the cytokine IL-1.

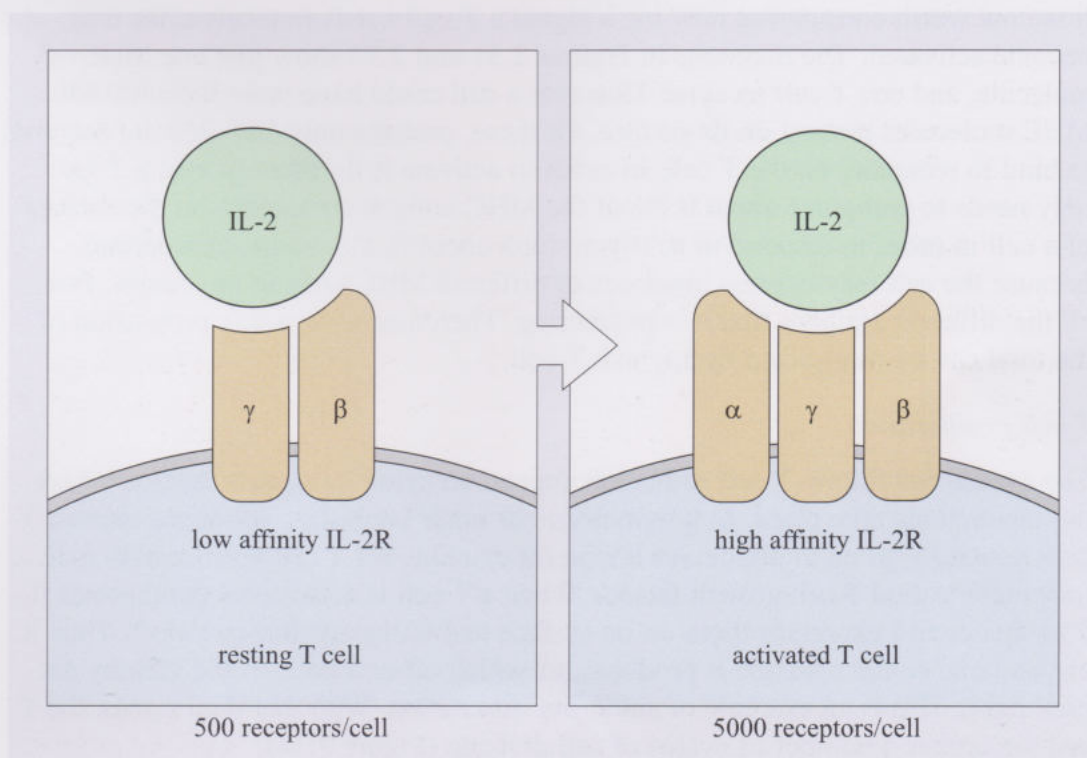


FIGURE 2.35

Resting T cells express small numbers of a low affinity IL-2 receptor consisting of a β chain and a signalling chain (γ). When a cell is activated it expresses an additional α chain, and upregulates (increases) the expression of the other chains, so that high levels of a high affinity receptor are expressed, and the cell is therefore much more readily stimulated by IL-2.

- ☐ Under what circumstances is IL-1 released? What consequence will IL-1 release have on T cell activation?
- ☒ IL-1 is an inflammatory cytokine released by activated macrophages, and sometimes by other cells in the tissues. Since IL-1 increases the expression of IL-2 receptors, this means that T cells will respond more readily to IL-2, and T cell activation and division is promoted.

T cell activation in tissues

If a T cell is activated in non-lymphoid tissues, it will still produce IL-2 and express IL-2 receptors, but the level of cell division is very limited. On the other hand, in non-lymphoid tissues the other cytokines produced by the T cells such as IFN γ are very important in orchestrating the immune response to infection.

- ☐ You have now encountered at least three different actions of IFN γ . Can you recall them?
- ☒ Interferon induces adhesion molecules and chemokines required for leukocyte migration into tissues. It causes many cell types to increase their expression of MHC molecules, so that they can present antigen more effectively. It activates macrophages to destroy pathogens they have phagocytosed. When a cytokine has several different distinct actions like this, we say that it is *pleiotropic*.

Control of T cell division

Clearly a T cell cannot go on dividing forever. T cells that have undergone several rounds of cell division fall back into resting phase, unless they are once more stimulated by antigen, presented by an antigen-presenting cell. This is very logical. The immune response is driven by lymphocytes responding to antigen. When the source of the infection and the antigens are gone, the response is no longer needed and cell division stops.

Costimulation, or more precisely the lack of it, determines whether T cells will fall back into resting phase. As a T cell is activated, the CD28 molecule interacts with B7 on the antigen-presenting cell. After a number of rounds of cell division, if the cell has not been restimulated with antigen, the CD28 is replaced with an analogous molecule, CTLA-4, which also binds to B7, but with higher affinity than CD28. So CD28 cannot engage B7 and therefore the T cell does not receive its costimulatory signal. Moreover, the CTLA-4 transmits an inhibitory signal to the T cells (Figure 2.36).

Antigen presentation by non-classical MHC molecules

Antigen presentation mediated by MHC class I and class II molecules has been understood since the early 1980s, but as scientists started to unravel the genome, it became clear that there were many other molecules that resembled MHC class I molecules, for which the functions were often unclear. These are sometimes called non-classical MHC class I molecules, to distinguish them from those encoded by the HLA-A, -B and -C loci, which we have been discussing above. Some of these molecules are encoded in the major histocompatibility complex, but others are encoded in odd places throughout the genome. Interestingly, however, some of these molecules can also present antigen. In particular, one set of molecules called CD1 has a very deep, hydrophobic antigen-binding groove, and they are able to bind to lipid or glycolipid antigens. Long acyl groups can occupy the deep groove,

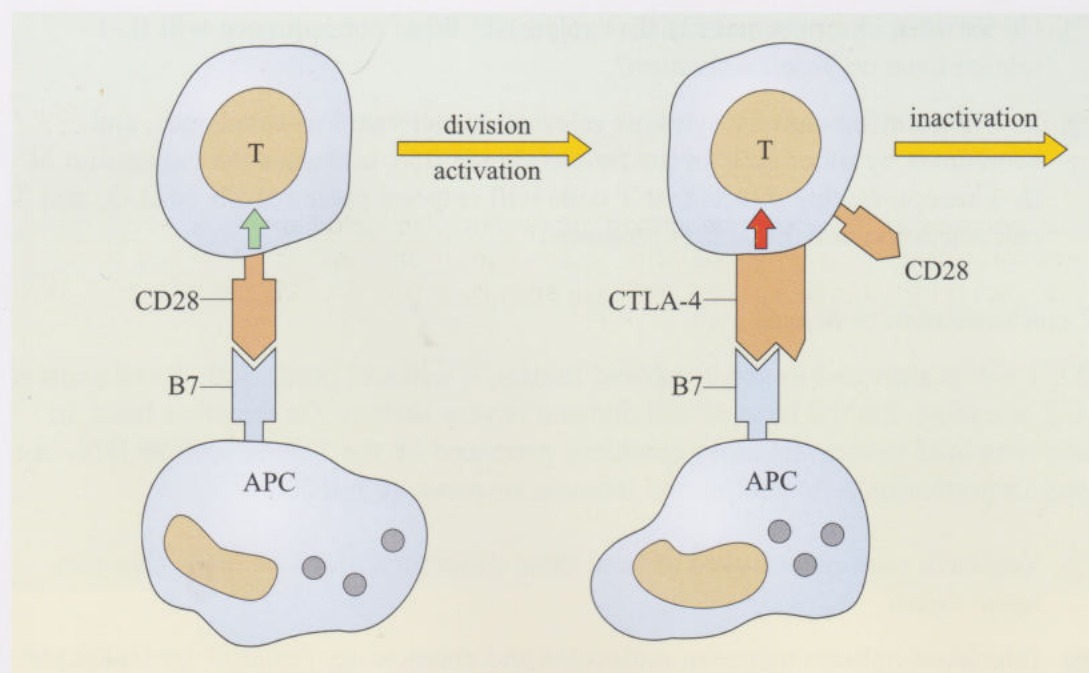


FIGURE 2.36

Ligation of CD28 on T cells leads to their activation. After several rounds of cell division they express CTLA-4 which has a higher affinity for B7 than CD28 and inhibits further cell division.

leaving other groups such as carbohydrate exposed to be recognized by the T cell receptor. In addition, one member of this family of molecules is able to bind lipo-arabinomannan.

- ☐ Where might lipo-arabinomannan come from?
- ☒ It is a component of the cell wall of mycobacteria (see Book 2, Section 2.2 and Figure 2.9).

Thus, it seems that whereas the standard MHC class I and class II molecules present polypeptide antigens, some of the non-classical MHC molecules, present other types of antigen.

The role of infection in antigen presentation

Before leaving the subject of antigen presentation, it is worth reviewing some of the ways in which infection can affect the process – remember that antigen presentation goes on all the time, regardless of whether infection is present. We have noted at several points that components of bacteria enhance antigen presentation as they can upregulate (increase) the expression of costimulatory molecules. In particular, macrophages have receptors for lipopolysaccharides (LPS) and various carbohydrates and glycolipids, which allow them to recognize bacterial and fungal components. It has long been known that components of bacteria can enhance immune responses. Such components are called **adjuvants** and a molecule which enhances a response, is said to have adjuvant activity. For example, when trying to induce a strong antibody response, immunologists sometimes inject the antigen into an experimental animal together with heat-killed mycobacteria. We can now relate the actions of these bacterial adjuvants to their ability to activate macrophages and promote costimulation and antigen presentation.

The simple pattern recognition systems used by macrophages predate the highly specific recognition systems of lymphocytes by many millions of years. Nevertheless, the original recognition systems now work in concert with the more recently evolved adaptive immune system to provide both stimulation and costimulation as joint elements of antigen presentation.

2.9.2 Antigen presentation (CD1)

You should now work through the topic on CD1 entitled *Antigen processing and presentation*, which is accessed by the icon of a macrophage presenting antigen to a T cell. This covers the area of antigen presentation by macrophages to Th1 cells, described above, but also includes antigen presentation by B cells to Th2 cells, a very similar process. Take this as an introduction to the subject because we shall return to T cell/B cell interactions later in the book. Also, you will see that the description of antigen presentation to cytotoxic T cells goes on to introduce the mechanism of cytotoxicity. This too is an area we shall return to later.

As you work through this topic, check the background screens on 'Antigen processing and presentation', 'The MHC-peptide binding site' and the class I and class II antigen presentation pathways. These will reinforce the material in the text. In addition, see the 3-dimensional molecular models of MHC class I and class II molecules, and try to identify the antigen-binding site, with its characteristic α -helices and β -pleated sheet base.

After you have completed the topic, you should be able to answer Questions 1–8 in the self-assessment section.

You should spend about 60–90 minutes on this activity.



2.10 Antibody-mediated immune responses

2.10.1 T-independent antibody responses

There are a few antigens that can stimulate B cells directly, causing them to divide and differentiate into antibody-forming plasma cells. These antigens are typically large polymeric molecules, with arrays of repeated epitopes, e.g. bacterial carbohydrates. They activate the B cells by cross-linking their surface receptors (Figure 2.37). Such antigens typically induce the formation of IgM antibodies that are of low affinity. Moreover, unlike the response to most antigens, they do not switch to IgG production (see Figure 2.6).

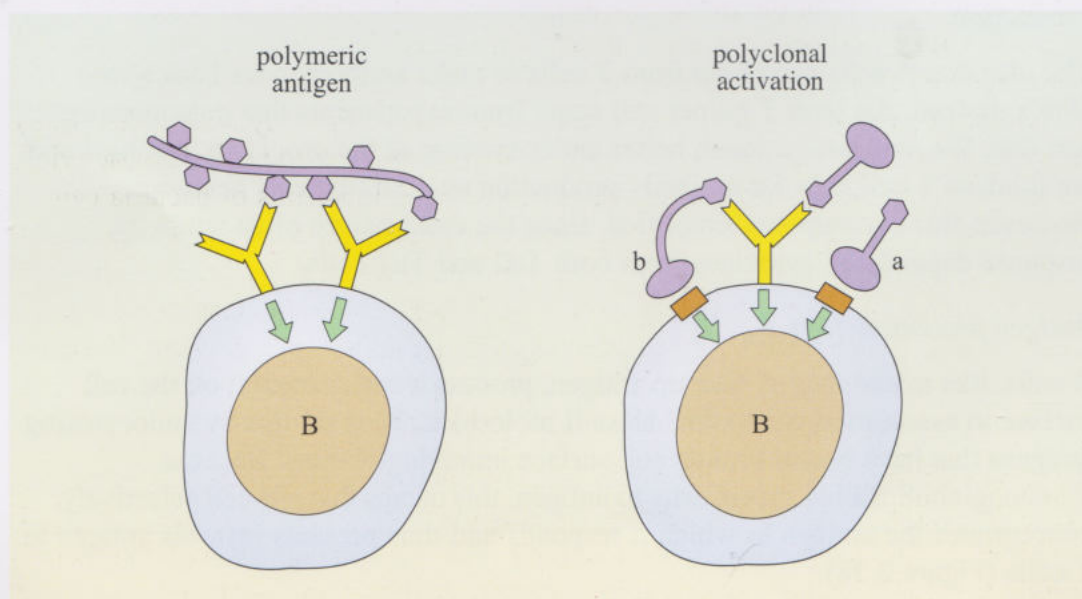


FIGURE 2.37

Two ways in which T-independent antigens can activate B cells are shown. Large polymeric antigens can cross-link the B cell's antigen receptors, which activates the B cell. Other antigens (polyclonal activators) are intrinsically biologically active and also act on other receptors on the B cell surface (a) to cause cell activation. At high doses, such molecules activate many clones of B cells, regardless of their antigen specificity. They are thus called polyclonal activators. At lower doses, B cells that have a B cell receptor for such antigens will concentrate the polyclonal activator to their cell surface and will then be triggered by it (b).

- What advantage might there be in producing low affinity IgM antibodies? Could they serve any function in protection against infection?
- Although they are of low affinity they are usually of high avidity, because they are pentamers with ten binding sites and so can bind to antigen via a number of epitopes. IgM antibodies are produced early in immune responses, and they activate complement very efficiently, so would be particularly useful in the early stages of an infection (see Sections 2.1.4 and 2.3.2).

B cells that respond to such polymeric antigens do not require help from T cells in order to differentiate and synthesize secreted antibodies. They are therefore called **T-independent antigens**, to distinguish them from **T-dependent antigens**, which require both T cells and B cells to recognize the antigen. There are several important differences between T-dependent and T-independent antigens, listed in Table 2.2. Notice in particular that T-dependent antigens induce high affinity IgG, IgA and IgE antibody production. In other words, T cells are required for antibody class switching. (See Section 2.1.1 for a description of the functions of different antibody classes.)

TABLE 2.2 Properties of T-dependent and T-independent antigens compared.

	T-dependent	T-independent
Antigens	monomeric	polymeric or polyclonal activator
	readily degraded and processed	resistant to degradation
Antibody produced	IgG, IgA, IgE, IgM high affinity	IgM low affinity
Examples	tetanus toxin	polymeric bacterial carbohydrates
	influenza haemagglutinin	lipopolysaccharide (LPS)

2.10.2 T-dependent antibody responses

We shall now look at how T cells interact with B cells in controlling antibody production.

The idea that B cells need help from T cells to make antibody goes back to the 1960s. Indeed, the term T helper cell came from experiments that demonstrated just this. We now have a much better understanding of the processes involved and we think of T cell help for antibody production as a Th2-type of response. However, this is somewhat simplified, since the exact nature of an antibody response depends on cytokines from both Th2 and Th1 cells.

Antigen presentation by B cells

B cells, like macrophages, take up antigen, process it and present it on the cell surface in association with MHC class II molecules. They do this by endocytosing antigens that have bound to their cell surface immunoglobulins. Because immunoglobulins bind specifically to antigen, this means that a B cell selectively concentrates the antigen to which it responds and thus presents just this antigen to T cells (Figure 2.38).

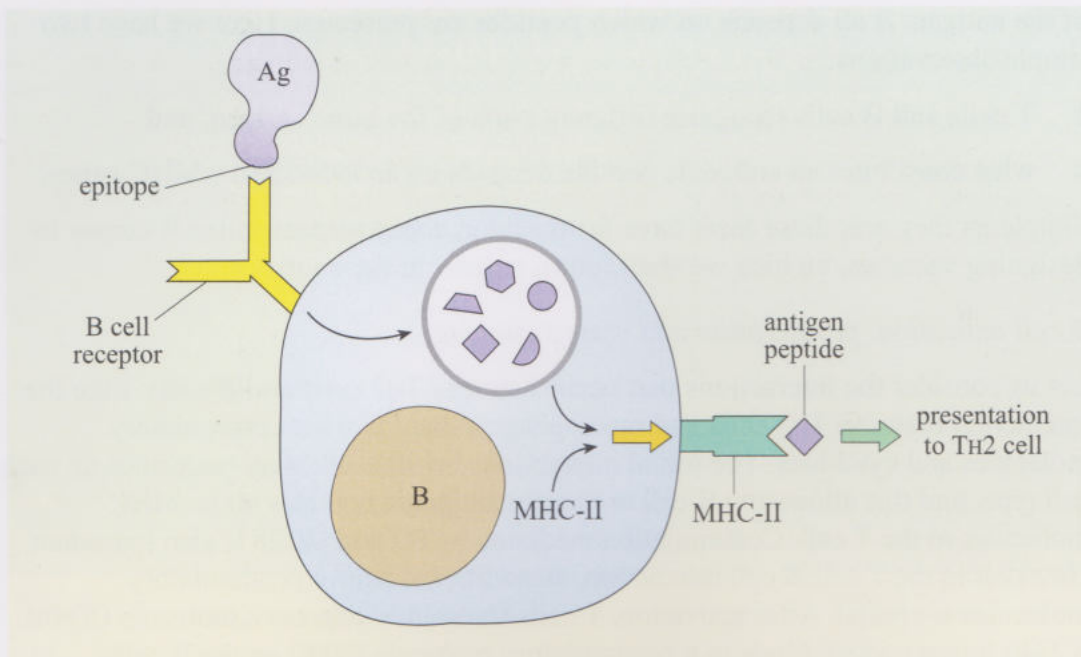


FIGURE 2.38 B cells bind antigen (Ag) via their surface immunoglobulin, B cell receptor. The antigens are internalized and processed and combined with MHC class II molecules to be presented to Th2 cells. Notice that the epitope recognized by the B cell is not usually the same portion of the antigen as the peptide that will be presented to the T cell.

- ☐ How do B cells and macrophages differ in the antigens that they present?
- ☒ B cells and macrophages both present the antigens which they have taken up. A macrophage has many receptors, which allow it to take up a wide variety of antigens, such as bacteria and fungi, microorganisms that have been opsonized by antibody and complement as well as immune complexes. Therefore a macrophage can present many different antigens. In contrast, the antibody on the surface of a B cell binds to just one antigen and so each B cell only presents one antigen.

B cells process antigen in a similar way to macrophages, that is they break it down into fragments which are then combined with MHC molecules in the MHC compartment (see Figure 2.32).

- ☐ What determines whether a fragment of an antigen can be presented to the T cell?
- ☒ The MHC class II molecule. If a peptide can bind in the groove on the class II molecule, then it can be presented. Antigenic peptides form non-covalent bonds with amino acid residues lining the groove and so the structure of the MHC class II molecule is critical (see Section 2.9.1). As MHC molecules are structurally very variable, they will differ in what antigen peptides they can present. This affects the ability to make an immune response and consequently an individual's susceptibility to particular diseases.

One contrast in the way in which cells process and present antigen is that B cells recognize one part of the antigen (the epitope), while T cells (recognising the same antigen) often recognize an antigenic peptide, and this is usually a different region

of the antigen. It all depends on which peptides are presented. Here we have two simple observations:

- 1 T cells and B cells recognize different parts of the same antigen; and
- 2 what constitutes an antigenic peptide depends on an individual's MHC genes.

Simple as they are, these facts have far-reaching consequences when it comes to designing vaccines, an idea we shall return to later in the course.

B cell activation, proliferation and class switching

Let us consider the interactions that occur between Th2 cells and B cells. Like the interaction between Th1 cells and macrophages, this involves costimulatory molecules and cytokines. The initial interactions involve adhesion molecules on each cell type, and this allows the B cell to present antigenic peptides on its MHC molecules to the T cell. Costimulation mediated by B7 and CD28 is also important. However in the T cell/B cell interaction, an additional pair of costimulatory molecules is crucial. After activation, T cells transiently express a molecule CD40L (CD40 ligand) which binds to a costimulatory molecule CD40 on the B cells. Ligation of CD40 sends a strong activation signal to the B cell and induces it to express B7, which acts to costimulate the T cell. Thus there is a mutual activation between the two cell types (Figure 2.39).

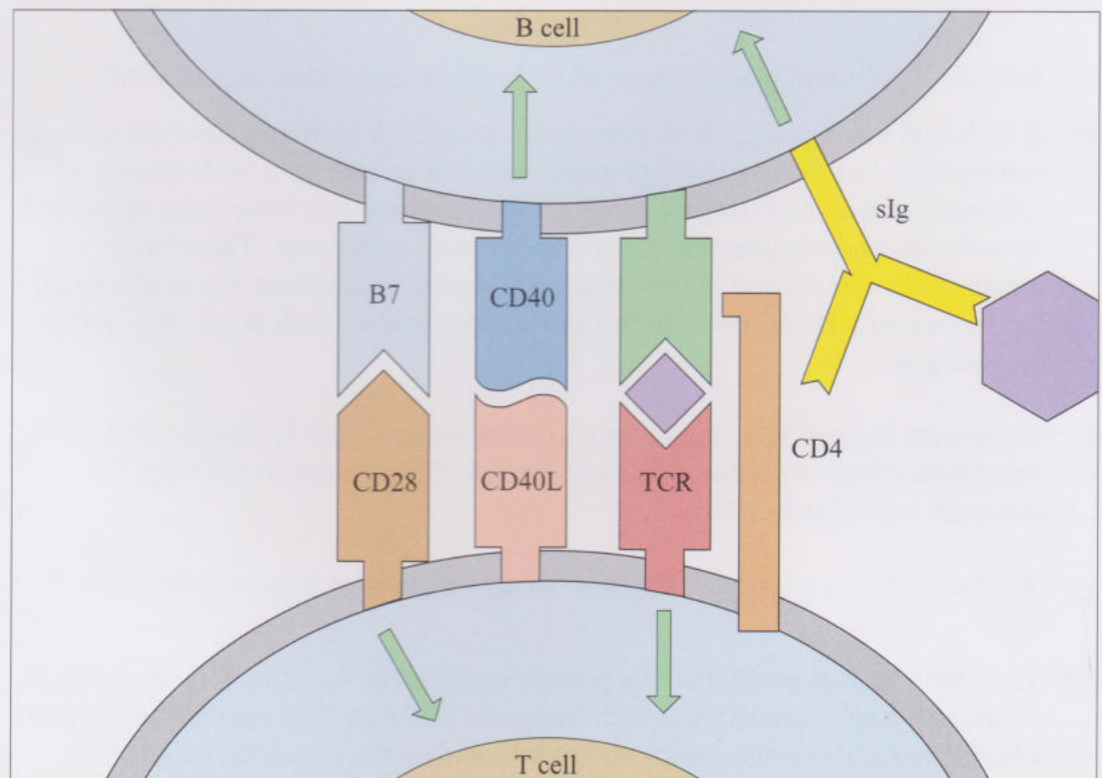


FIGURE 2.39
When B cells interact with T cells they receive a costimulatory signal from CD40, which together with a signal from their antigen receptor (sIg) leads to B cell activation and induces synthesis of B7. The T cell is in turn activated by a signal from its T cell receptor and from the B7 molecule acting on CD28.

One event that occurs as an antibody response develops is the switch from production of IgM, to other antibody classes IgG, IgE and IgA, and this is called **class switching**. The interaction between Th2 cells and B cells is essential both for the development of the antibody response and for the class switch which occurs during a secondary immune response. This can be demonstrated in rare individuals who genetically lack CD40L. They have a very high level of IgM in the blood, but low levels of IgG, IgA and IgE. Because the T cells cannot pass the costimulatory

signal to the B cells, the B cells fail to switch from IgM production to other classes. A new idea has been introduced here: individual B cells can switch from producing IgM to IgG, and this cellular event underlies the switch in antibody classes seen in an immune response.

One other thing is notable in individuals who lack CD40L – their lymph nodes lack germinal centres (see Figure 1.5). This gives us an indication that class switching occurs in the germinal centres of lymphoid tissues, and this does indeed turn out to be the case.

Following T cell/B cell interaction, cytokines come into play. Particularly important is IL-4, formerly called B cell growth factor, which is released by T_H2 cells. Activated B cells express an IL-4 receptor and respond to IL-4 by dividing. Other cytokines released by T_H2 cells including IL-5, IL-6 and IL-13 promote differentiation of the B cells into antibody forming cells and ultimately into plasma cells and memory B cells (Figure 2.40).

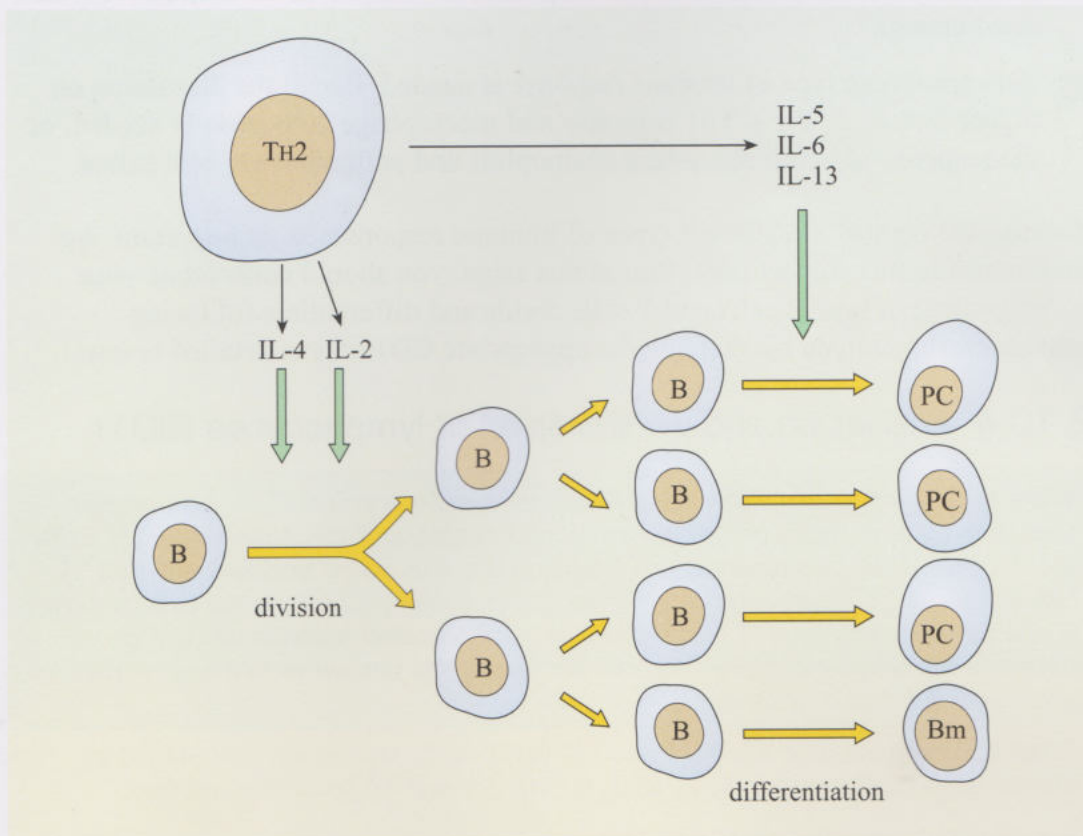


FIGURE 2.40 Interleukin 4 produced by T_H2 cells induces the division of B cells. IL-2 can also potentiate B cell division. Other cytokines, including IL-5, IL-6 and IL-13 promote differentiation into antibody-secreting plasma cells (PC) and memory B cells (Bm).

- ☐ What is the difference between the antibody that is initially produced by the B cell and the antibody that is produced by the plasma cell?
- ☒ The B cell's antibody is a cell surface molecule with a transmembrane region that acts as a receptor for antigen. The plasma cell produces a secreted antibody that lacks the transmembrane region, but is in other respects similar to the cell surface form (see Section 1.2.1).

The cytokines not only promote the differentiation of B cells, but they also can affect the type of class switch that the B cell makes. For example IL-5 tends to promote switching from IgM to IgE production. (We have already described another function for IL-5 – the release of eosinophils into the blood.) Because cytokines like IL-4, IL-5, IL-6 and IL-13 are produced by Th2 cells, and because they promote the production of antibodies (and sometimes also allergic reactions) this type of response is often called a Th2-type of response.

2.10.3 Th1 versus Th2 responses

How does the body 'know' when to make a Th1 response or a Th2 response? This partly depends on the genetic make-up of the individual and partly relates to the way in which the immune system first encounters the antigen. This in turn is related to how the antigen is first presented to the different populations of T cells.

- ☐ If we are designing a vaccine for a pathogen, does it matter whether we produce a Th1 response or a Th2 response, or is any type of immune response good enough?
- ☒ An appropriate type of immune response is needed. Recall the discussion on mycobacteria, where a Th1 response and macrophage activation is needed, or the response to helminths where eosinophils and antibodies are best suited.

Because the control of different types of immune responses is so important, we shall return to this subject later. But, at this stage, you should consolidate your understanding of how B cells and T cells divide and differentiate following activation with antigen by studying the appropriate CD1 unit as detailed below.

2.10.4 Activation and proliferation of lymphocytes (CD1)



Work through the animation entitled *Clonal proliferation* on CD1. This brings together a lot of material on antigen presentation and the activation of B cells and T cells. Useful summaries are found on the expansion screens entitled 'B cell activation', 'B cell activation – role of cytokines', 'Cytokine receptors during B cell development' and 'T cell activation'. The detailed material on the B cell coreceptor complex and the intracellular signalling pathways are not needed to understand *Infectious Disease*.

After you have studied the text and CD-ROM up to this point, you should be able to answer Questions 1, 2, 3, 6, 8, 10, 11 and 14 from this section.

Spend about one hour on this activity.

2.10.5 Development of the antibody response

So far, we have noted that T cells help B cells to make antibodies, that they direct antibody class switching and that the events which underlie the development of the antibody response take place in lymphoid tissues.

This section and the CD-ROM unit that follow it are intended to give you some insight into these events. This is a marvellous example of how we can understand a process like antibody production at different levels:

- At the level of an individual: antibodies in serum switch from low affinity IgM to high affinity IgG during the response to a T-dependent antigen as in Figure 2.6.

- At the cellular level: individual B cells switch from producing IgM to IgG, IgA or IgE.
- At the molecular level: the immunoglobulin genes that a B cell uses to make its antibodies are switched as the B cell matures.

Class switching

In order to understand the processes of immunoglobulin class switching, we need to look at the genes that encode the immunoglobulin heavy chain (Figure 2.41). Look first at the gene for IgM. Like most mammalian genes it consists of a number of coding segments (exons) which are separated by non-coding segments (introns). The coding and non-coding segments are transcribed as one long piece of RNA that is then spliced, to bring the coding segments together forming messenger RNA (mRNA). The variable domain of the heavy chain is encoded by one exon (VDJ), and each of the constant domains is encoded by another exon. There are also exons for the transmembrane segments and intracytoplasmic regions of cell surface antibody. The exon encoding the variable domain is formed during B cell development in the bone marrow, a process which involves reassortment and recombination of several gene segments, referred to as 'V', 'D' and 'J'. We shall not go into this process, but just take note that the end result is that the gene that encodes the variable domain of a heavy chain is often called a VDJ gene segment.

Remind yourself of the domain structure of antibodies (Figure 2.1) in order to follow the descriptions in this section.

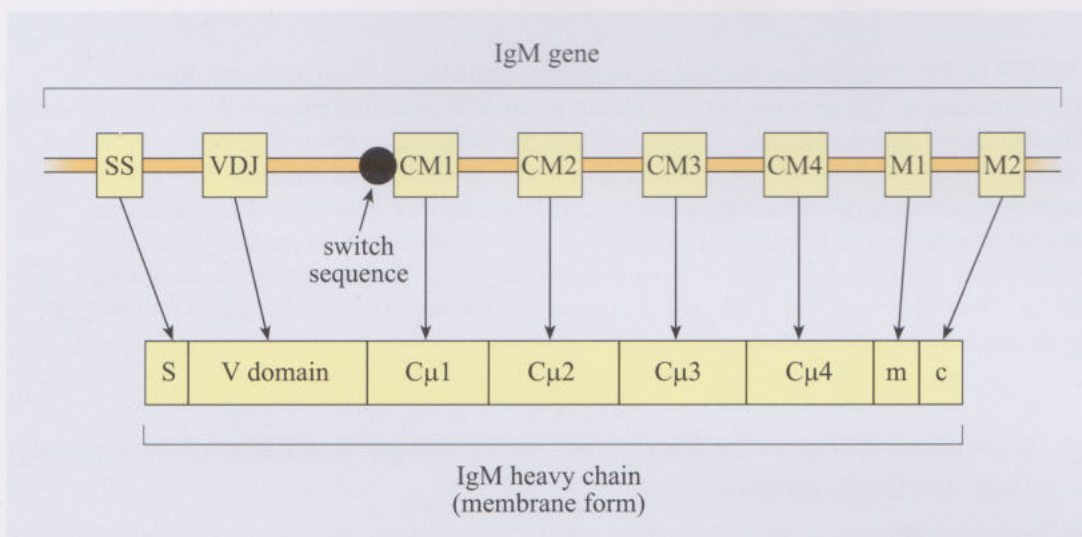


FIGURE 2.41 The IgM gene is divided into exons (labelled) separated by introns. After the gene has been transcribed, the RNA (not shown) is processed to bring the segments together which will encode heavy chain protein. The diagram shows which part of the gene encodes which part of the protein. Generally there is one exon for each domain. A small exon (ss) encodes a signal sequence that directs the translation of the IgM heavy chain into the endoplasmic reticulum. Next come exons encoding the variable and constant domains. Two other small exons (M1 and M2) encode the transmembrane segment and intracytoplasmic portions of the polypeptide (m.c.). A switch sequence in the DNA is important in switching between production of different antibody classes. The diagram of the gene is not to scale – the non-coding sections are much longer than the exons.

Further downstream of the IgM exons on the same chromosome are genes for the constant domains of the heavy chains of all the other immunoglobulin classes (Figure 2.42). Each of these sets of genes is preceded by a switching sequence.

Notice, however, that there is only one VDJ exon encoding the variable domain. In naïve B cells, the VDJ exon is transcribed together with the exons for the IgM constant domains, so that IgM is produced. As the B cell develops and with T cell help, a rearrangement of the genes takes place so that genes for one of the other immunoglobulin classes are moved up to replace the IgM exons. This means that the VDJ gene is now transcribed together with constant domain genes for one of the other immunoglobulin classes (Figure 2.42). The intervening genes, including the IgM genes, are lost during this process. This is the genetic event which underlies antibody class-switching by the cell and which underlies class-switching in an immune response.

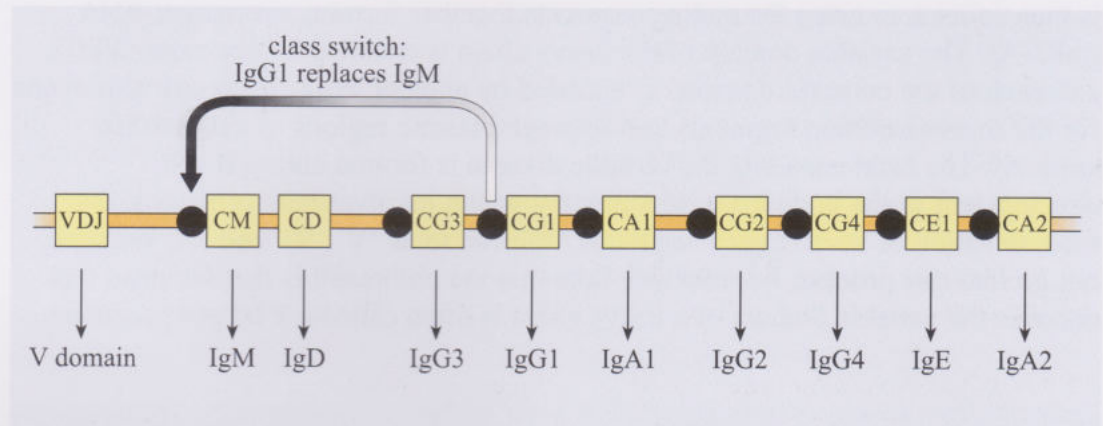


FIGURE 2.42 The genes of the human immunoglobulin heavy chain locus are shown diagrammatically. The gene for IgM lies closest to the VDJ gene that encodes the V domain. Genes for other heavy chains of immunoglobulin classes lie downstream. (Note that each of these loci will contain several exons as indicated in Figure 2.41.) When a B cell switches the class of antibody it produces, the switching sequence preceding the new gene recombines with the switching sequence preceding the IgM gene, bringing it next to the VDJ gene segment. In this example a switch from IgM to IgG1 production is illustrated. The intervening genes for IgM, IgD and IgG3 would be lost. The VDJ gene is now transcribed together with the genes for the IgG1 heavy chain rather than with those for IgM.

Because there is a separate gene for each of the antibody subclasses, these are genetic isotypes. Distinguish *isotypes*, where every member of the species possesses a gene for the variant, from *allotypes*, variants present at one gene locus in some individuals and not others.

- ☐ What effect will class-switching have on the antigen specificity of the antibody which the B cell produces?
- ☒ There is no effect. The specificity of the antibody is determined by the amino acid sequence in the variable domains which is encoded by the VDJ gene segment. Since this segment stays the same during class switching, there will be no change in the antibody specificity.

Affinity maturation

A second event that occurs during the development of an immune response is a progressive rise in the affinity of the antibodies, which is called **affinity maturation**. To understand how this process occurs at a cellular level, we need to look into what is happening in the lymphoid tissue. Interestingly, the processes that occur at the cellular level involve mutation and diversification of B cells, followed by selection of the fittest B cells. Does this sequence look familiar? In fact it is wholly analogous to the processes underlying evolution on the grand scale. However in this case, the 'natural selection' of the antibody response takes place within our bodies over the period of a few days.

As we noted above, class-switching happens in the germinal centres of the spleen, lymph nodes, Peyer's patches and other lymphoid tissues. As an immune response develops, individual B cells enter the germinal centre and undergo very rapid cell division. As they do this, a mutation mechanism is activated which affects just the region around the immunoglobulin heavy chain genes. The mechanism for the increased localized mutation is not known, but it is so high, that if it occurred throughout the whole of the genome many of the cell's essential enzymes would be rendered non-functional due to damaging mutations. Each of the B cell progeny from a single precursor will develop different mutations in their heavy chain genes. In other words, the B cells from the same clone will diversify slightly with respect to the antibodies they produce.

- ☐ What effect will the mutations have on the affinity of the antibodies produced by a clone of B cells derived from a single precursor?
- ☒ Most antibodies will be unaffected. A few antibodies will have mutations in their hypervariable regions. In many cases this will mean that the antibody binds less well to the antigen (lower affinity). In the worst case, the antibody will be completely unable to bind to its epitope. Just occasionally, however, the mutated antibody will have a better fit for the epitope than the original did, i.e. the mutated antibody will have a higher affinity for the antigen.

At this stage a selection process comes into play. Remember that B cells only receive help from T cells if they present antigen to the T cells. The B cells in our scenario have diversified, some with high and some with low affinity antibodies as their antigen receptors, but any cells that have lost their ability to bind the antigen cannot take it up. Consequently they have no antigen to present to the T cells and they do not receive T cell help. The situation is more critical than this. In the lymphoid tissues the quantity of antigen available is limiting, so only B cells with the highest affinity antibodies are able to take up sufficient to present to the T cells and get help (Figure 2.43). In other words, only B cells that have developed high affinity antibodies get T cell help.

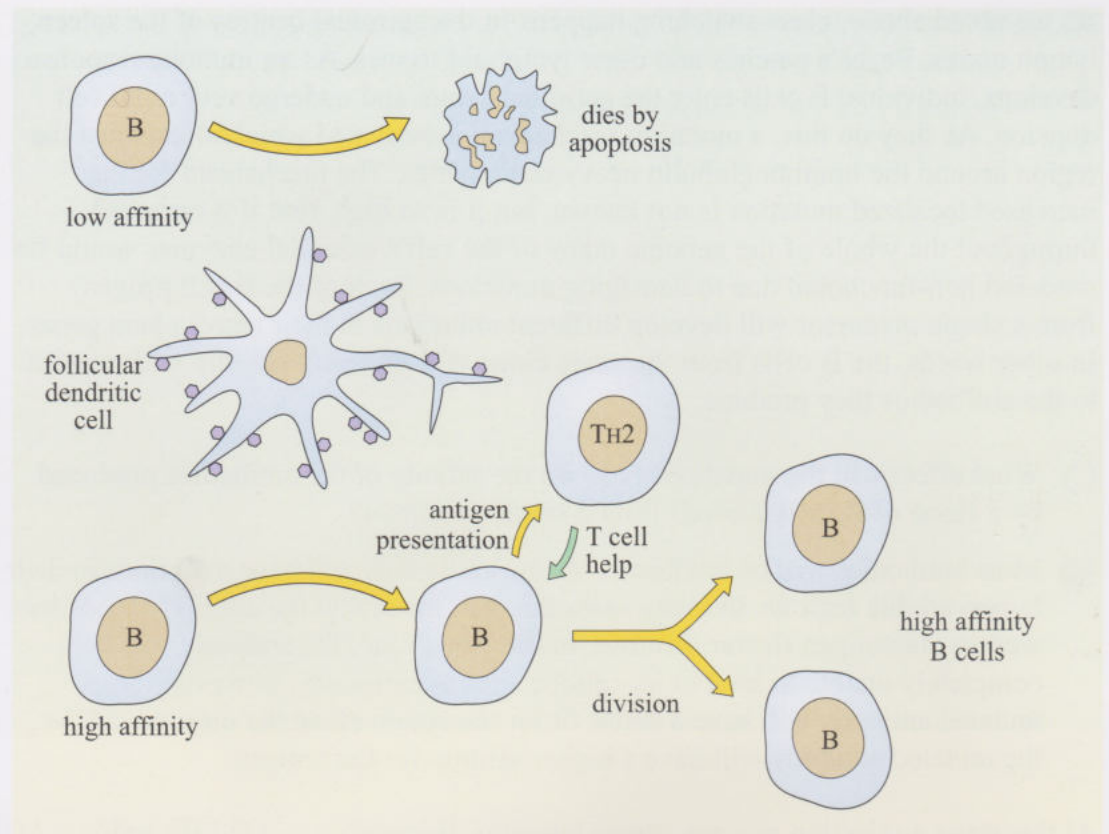
The way this works is as follows: when the B cells enter the germinal centre and divide, they become programmed to die (by apoptosis) *unless* they are rescued by interaction with helper T cells. The molecular signals which are involved in the rescue process include the costimulatory molecule CD40, and the result is that only high affinity B cells survive passage through the germinal centre and go on to divide and differentiate. The other B cells just die and are phagocytosed by macrophages.

The overall effect of the selection process is that high affinity B cells develop during an immune response and so the affinity of the antibodies produced, increases as the immune response progresses (see Figure 2.6).

You have now seen two processes occurring in the germinal centre – class-switching and the development of high affinity B cells. These processes appear to happen synchronously, although they are distinct. As T cells promote class switching, you can now see why T independent antigens do not induce IgG responses, while T-dependent antigens do. Also, as class switching is associated with the increase in affinity, it is clear why IgG antibodies tend to be of higher affinity than IgM.

FIGURE 2.43

In germinal centres follicular dendritic cells hold stores of antigen which may be taken up by B cells. Low affinity B cells do not take up and process sufficient antigen and die by apoptosis. High affinity B cells take up antigen, present it to T cells and receive T cell help for division and differentiation. Thus populations of B cells with high affinity antigen receptors are selectively expanded.



While we understand class-switching in broad outline, many important details are still being investigated. In particular, we know for example that B cells in mucosal lymphoid tissues tend to switch to IgA production, but how the T cells promote a switch to this class rather than another class is only partly understood. As you will see later, cytokines can have a role, but this is not the whole story.

2.10.6 Development of antibody classes (CD1)



Now you should work through the CD1 topic entitled *Antibody classes* which is accessed via the Navihedron icon of a dimeric IgA molecule. This will explain the processes of affinity maturation and class-switching dynamically. As you go through the topic, concentrate on the cellular events that take place in the lymphoid tissues, since these have received less coverage in the text above. In particular, take note of the role of the follicular dendritic cell and look up the expansion screens on 'Follicular dendritic cells', 'Lymphoid follicles' and 'Germinal centres'.

The second part of the animation is concerned with the functions of different antibody classes, which should be familiar to you by now. So, you should be able to answer all of the self-assessment questions (1–8) associated with the unit.

Spend about one hour on this topic.

2.11 Immunological memory

Memory is one of the key features of an adaptive immune response and is seen in both B cells and T cells. This concept is so important, in relation to immunity and vaccination against disease, that we should briefly review the cellular basis of **immunological memory**.

- ☐ From your general experience of infections and vaccination regimes, how long you do suppose that immunological memory lasts – one year, five years, fifty years?
- ☒ Typically immunological memory is longlasting, perhaps fifty years, but it depends on the pathogen. For some diseases such as rubella, immunity can be lifelong; in contrast, the recommendation for cholera immunization is for a booster every six months.

Several components contribute to the enhanced immune response that occurs following a second encounter with an antigen or a pathogen. Most important is the increase in the numbers of cells that can recognize the antigen, as a result of clonal expansion in the primary immune response. Following a first infection or vaccination many of the cells that react to the antigen, terminally differentiate and subsequently die. But a proportion of these develop into **memory cells**. These become distributed throughout the secondary lymphoid tissues and are available to be reactivated if the antigen returns. Clonal expansion applies to both B cells and T cells and it means that the pool of cells available to respond in a secondary response is much larger than in the primary immune response. Typically the frequency of specific T cells will be enhanced 10–100-fold after the primary response.

The lifespan of memory cells can be several decades, and this is reflected in the duration of immunological memory. For example, small island communities often have epidemic patterns of diseases such as measles. During an outbreak, most of the population catches the disease, and then becomes immune, so that the disease dies out in the community until sufficient children have been born to provide a new susceptible population. In such small communities it could often be 50 years between outbreaks, and an outbreak would only occur if a boat with an infected person had landed on the island. In such cases it is notable that the very old people would not catch the new infection, because they had been infected when they were children. This means that their immunity had lasted a lifetime. Since the virus had been absent during the intervening years, there was no opportunity for repeated stimulation of their immune system, and the immunological memory must have resided in the lymphocytes for the intervening years.

2.11.1 Memory cells

What is the nature of a memory cell? Most activated lymphocytes die within a few days or weeks of production. This is absolutely necessary, so that the overall population of lymphocytes remains more or less constant over a lifetime. Most activated B cells differentiate into plasma cells that die by apoptosis, and this is termed ‘terminal differentiation’. It is easy to distinguish plasma cells because of their distinctive shape and some characteristic surface markers. It is less easy to distinguish terminally-differentiated T cells. Nevertheless, it is inferred that most effector T cells also die after an immune response has subsided, leaving only a smaller memory population behind. The key to the great longevity of memory cells is that they do not undergo terminal differentiation. Memory B cells appear to produce a transcription repressor, which prevents the cell turning on genes that are required for differentiation into the plasma cell. Although the mechanism is not known, it is thought that memory T cells can also repress their terminal differentiation. In some ways, therefore, memory cells have the characteristics of stem cells, that is, they are able to renew themselves over long periods of time (Figure 2.44).

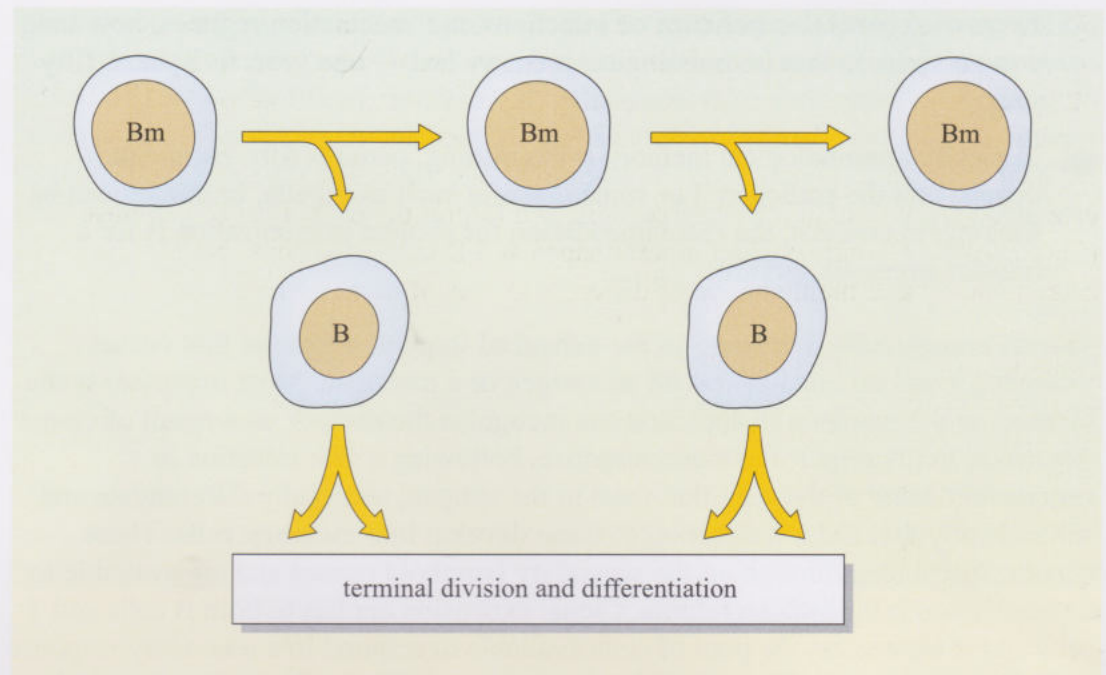


FIGURE 2.44 Long-lived memory B cells (Bm) are thought to have characteristics of stem cells in that they can undergo asymmetric division to produce B cells and further memory cells. In contrast, most effector lymphocytes undergo terminal differentiation and die within a few weeks or months.

As well as the increase in cell numbers and their long lifespan, memory cells have undergone a series of differentiation steps that make them more responsive on subsequent encounter with the antigen. For B cells, differentiation is characterized by the switch to production of IgG and IgA, and an increase in antibody affinity. Memory T cells have a different profile of surface molecules compared with naïve T cells. In particular, some adhesion molecules and costimulatory molecules are increased, which means that the cells respond more rapidly to a smaller antigenic signal. In addition, there are changes in one of the molecules which modulates the activation of the T cell receptor. Although some aspects of memory are understood, many of the molecular mechanisms are still under investigation. In summary, we can identify three underlying components of immunological memory:

- an increase in the number of cells that can recognize an antigen or pathogen;
- differentiation of cells so they can respond to it more quickly and effectively;
- production of a set of long-lived or self-renewing memory cells.

2.12 Th1 and Th2 responses

In Sections 2.9 and 2.10 we looked at different types of immune response: the cell-mediated response mediated by macrophages and Th1 cells, and the Th2-type response, which is characterized by antibody production. This section is concerned with aspects of the way these responses are controlled.

To begin with, we shall think of the two types of response as opposite ends of a spectrum, although this is somewhat simplistic and the idea will need refining later. We have already seen that cytokines are important in promoting the different types of immune response and that the different types of T cells produce distinct sets of

cytokines. (Table 2.3). Going beyond this we shall see that some of the cytokines produced by one cell type can inhibit another. In fact $\text{IFN}\gamma$ produced by Th1 cells inhibits the action of Th2 cells and one cytokine, IL-10, produced by Th2 cells, inhibits cytokine production by Th1 cells. In effect, the two types of T cell can work in a flip-flop mechanism – once an immune response of one type is established, it will tend to reinforce itself and inhibit the other type of response (Figure 2.45). Cytokines from macrophages and B cells also contribute to the establishment and maintenance of different modes of immune response.

TABLE 2.3 Cytokine production.

Th1 cell cytokine production	Th2 cell cytokine production
$\text{IFN-}\gamma$	
IL-2	IL-4
	IL-5
	IL-6
	IL-10
	IL-13
TNF- α	TNF- α
TNF- β	TNF- β

Cytokines produced by Th1 cells and Th2 cells are indicated. Some cytokines such as TNF, are produced by both cell types.

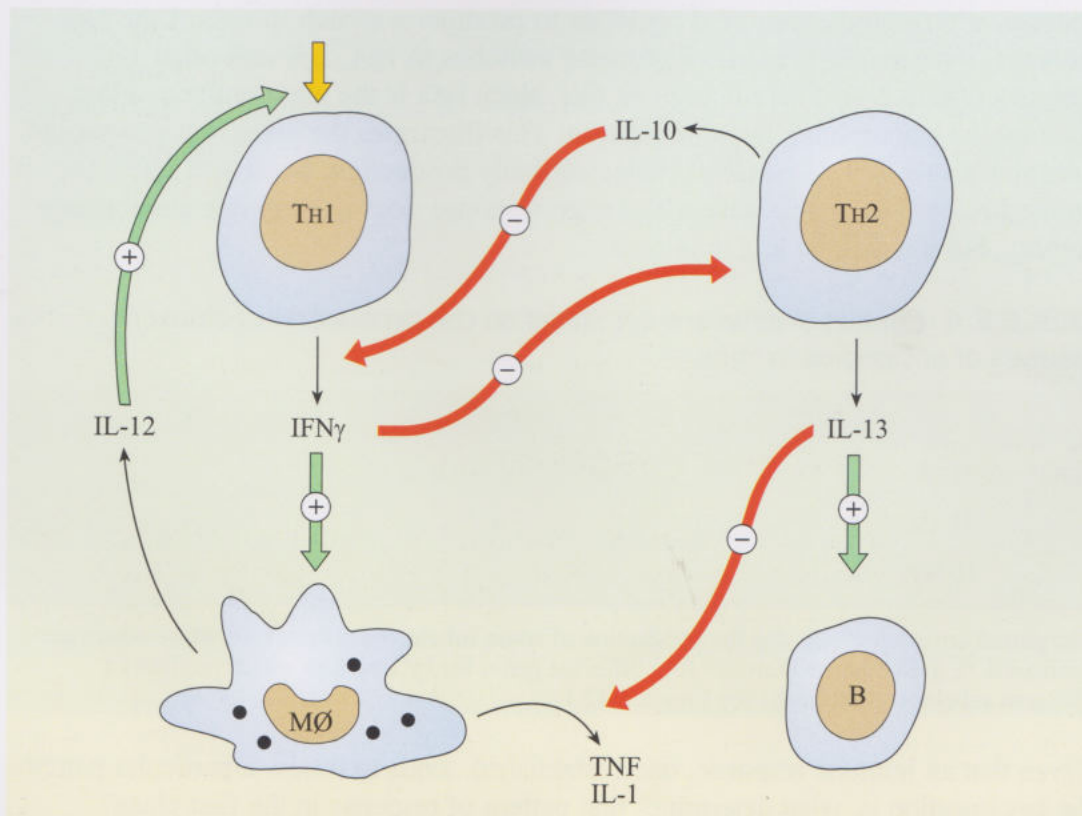


FIGURE 2.45

Regulation of the Th1 and Th2 response. Interferon- γ ($\text{IFN}\gamma$) produced by Th1 cells promotes macrophage activation but also suppresses division of Th2 cells. IL-12 produced by macrophages promotes differentiation of Th1 cells. Th2 cells produce IL-10, which suppresses synthesis of $\text{IFN-}\gamma$ and IL-13 which both promotes B cell division and inhibits production of inflammatory cytokines by macrophages. Thus both types of immune response can reinforce themselves and inhibit the other type of response.

2.12.1 Modes of immune response (CD1)



There is a short topic on CD1 entitled *Modes of immune response* that you should work through now. It is accessed via the Navihedron icon of two lymphocytes on a balance. This unit emphasizes the interactions that take place between T_H1 cells and T_H2 cells and the roles of cytokines in maintaining this balance. See the expansion screen on ' T_H1 and T_H2 -type immune responses' to consolidate your understanding of this area. Then look at the screen entitled 'Relative importance of antibody-dependent and antibody-independent responses in protozoal infections'. This gives examples of particular ways in which particular responses are best suited for dealing with specific infections.

After you have completed the topic, you should be able to answer Questions 1–4 and 6–8 associated with this section. You may find that some additional research is needed to answer all the subsections of the questions, in which case use the 'i' button from the questions to take you to the relevant expansion screens of background information.

Spend about 45 minutes on this activity.

2.12.2 Selection of the mode of response

The unit in *Immunology Interactive* sets out a case for two opposing types of immune response, with each one inhibiting the other. However, as you saw with bacterial and protozoal infections, the requirements of immune defence are not so clear cut. For example we need antibodies to opsonize bacteria for uptake by macrophages, but also need the $IFN\gamma$ to activate the macrophages to destroy the pathogens they have taken up. In fact cytokines can modulate the production of antibodies and affect the class that is produced. This has been most clearly seen in rodents, where production of $IFN\gamma$ tends to promote a switch to one of the IgG isotypes, whereas IL-4 and IL-5 promote switches to IgA, IgE and other IgG isotypes (Table 2.4). There is logic in this, since IgG is the main antibody class used by the macrophage for opsonization. This illustrates the point that you should not think that a T_H1 response excludes antibody production, but it will affect the antibodies produced. Likewise a T_H2 -type response does not exclude macrophage activity, but this will be less important.

TABLE 2.4 Effects of different cytokines on the production of different classes of antibodies in mice.

		IgG	IgA	IgE
T_H2	IL-4	$\uparrow\downarrow$		
	IL-5	-	$\downarrow$	$\uparrow$
T_H1	$IFN\gamma$	$\uparrow\downarrow$	$\downarrow$	$\downarrow$

The paired arrows indicate that the production of some subclasses is increased while others are decreased. (e.g. in humans there are four different genes for IgG, each of which produces a different subclass of antibody; see Figure 2.42.)

Given that an immune response, once established, tends to follow a particular pattern, the key question is, what determines that pattern of response in the first place?

Genetic effects

When immunologists first induced immune responses in inbred strains of animals, by injecting them with antigen preparations, it was often found that some strains produced a strong antibody response, while others produced a strong cell-mediated response. This implies that the genetic make-up of an individual has some role in determining the type of response they will produce. For example, an animal that produces a high level of IFN γ in response to pathogens, or responds readily to this cytokine, will tend to show a TH1-type of response. Conversely, an individual that produces a high level of corticosteroids (which suppress macrophage-mediated immunity) will more likely produce a TH2-type of response.

Humans, of course, are outbred and therefore genetically more diverse than the inbred strains of rodents in which these results show up clearly. Nevertheless, there is good evidence that the genetic diversity of humans has produced people who will tend to make antibody responses and others who produce cell-mediated responses when confronted with the same antigen or pathogen.

Antigen presentation

Another important factor in controlling the mode of immune response is the way in which antigen is first presented to the immune system. Take, for example, food. Every day we take in huge amounts of antigens in the form of food, but mostly we do not make immune reactions against food antigens. However, if we ingest a pathogenic bacteria we will make a response very quickly, even though the antigen load which the bacteria presents is much smaller.

- ☐ What reasons can explain why we make a strong response to a bacterium and no response to food antigens that may both be present in the gut at the same time?
- ☒ Bacteria are recognized and handled by, for example, macrophages that present antigen to T cells together with costimulatory signals, thereby inducing an immune response. (Recall that bacterial products and inflammatory cytokines induce costimulatory molecules on antigen-presenting cells.) Food antigens are not processed and presented by antigen-presenting cells in the same way as pathogens.

Antigen handling is therefore an important factor in determining whether we will develop an immune response at all. It also affects the type of response we develop. For example, an antigen injected into the skin will most often cause the production of an antibody response dominated by IgG. Conversely, if the same antigen is given as an aerosol into the nose, it can produce an IgA response. The way the antigen is handled in either case is different. The injection into the skin will lead to antigen going to local lymph nodes where it will be presented by dendritic cells, follicular dendritic cells and macrophages. The B cells in lymph nodes tend to produce IgG and the T cells in their germinal centres tend to promote this class-switch. In contrast, T cells in the mucosa-associated lymphoid tissues cause a high proportion of B cells to switch to IgA production. Moreover, IgA-producing B cells tend to localize in mucosal tissues, as they migrate through the body. This means that the site of entry of a pathogen will affect the type of immune response that it induces.

When administering vaccines, the way in which it is given (the route) can radically affect the type of immune response that develops.

- Why might it be detrimental to give a malaria vaccine as an aerosol preparation, but an advantage to administer an influenza vaccine in this way?
- Administering an aerosol vaccine is likely to promote a Th2 type of response with IgA antibody production. This is very useful against influenza, where the pathogen enters the body through mucous membranes. However IgA is of no particular value against malaria, which does not cross mucous membranes. Moreover, initiating a Th2 response may *inhibit* the Th1 response that is needed for protection against the malaria parasite.

2.13 Immune defences against viruses

Viruses present a particular set of problems for the immune system, and a number of immune defences have evolved to combat virus infections. When a virus is *outside* the cell, it can be handled in much the same way as any other antigen, that is, it can be opsonized by antibodies and complement, taken up by phagocytes and broken down by degradative enzymes. However, once a virus has established an infection *within* a cell, different defence mechanisms are needed. These include the cytotoxic T cells and natural killer (NK) cells, mentioned earlier. Also important are the interferons that can limit viral spread in the earliest phases of infection. Therefore the defences against viruses include different elements for dealing with the intracellular phase of infection and the extracellular phase (Figure 2.46). The relative importance of the various mechanisms depends on the virus and the type of infection it causes. For example yellow fever virus is injected by an insect bite directly into the blood, so IgA antibodies are less important than IgG, whereas infections with cold viruses are confined to the mucosal epithelium, where IgA is very important.

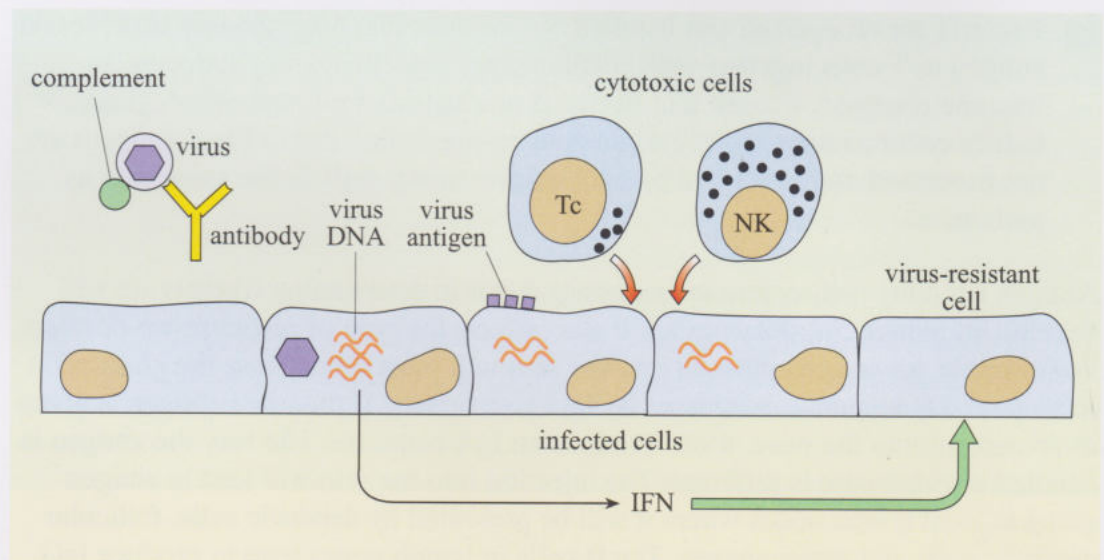


FIGURE 2.46 Overview of anti-viral defences. Antibodies binding to the virus surface can prevent it attaching to the viral receptor on the cell surface, and promote phagocytosis by cells with Fc receptors. Complement can also promote phagocytosis and causes damage to viral envelopes. Once a virus has infected a cell, the release of interferons (IFN) signals to neighbouring cells to induce virus resistance. If cells become infected they are susceptible to killing by cytotoxic T (Tc) cells and natural killer (NK) cells.

2.13.1 Interferons

Although we have already discussed the role of IFN γ at length, this has been in respect of its role in mediating the immune response, promoting leukocyte migration across endothelium and activating macrophages. However interferons were first identified because of their role in *interfering* with virus replication. This group of cytokines consists of three members IFN α , IFN β and IFN γ , and they are important in slowing virus spread during the earliest phases of viral infection. This is critical since viruses can replicate very rapidly and the immune response takes several days to get going, so anything that can buy time for the immune system is valuable.

IFN α is produced by leukocytes and IFN β by fibroblasts and epithelial cells. These two interferons act on a receptor that is widely distributed on most cells in the body. IFN γ , which is produced by activated T cells and NK cells acts on a different receptor, which is also widely distributed.

Interferons α and β are also produced by cells that have become infected by virus. Although this does not help the original cell it does signal to neighbouring cells to alert them that a virus is present. A cell that receives a signal from IFN responds by synthesising a group of antiviral proteins. Many of these stay in an inactive state unless a virus enters the cell and attempts to go through its replication cycle. As viruses replicate they may produce molecules that are specific to replicating viruses (e.g. double-stranded RNA), and that are able to activate the anti-viral proteins. Once activated, the proteins have a number of functions, which include shutting down protein synthesis within the cell (Figure 2.47). For example, one protein accelerates the breakdown of messenger RNA, whilst another inactivates a factor that is needed to initiate translation of mRNA.

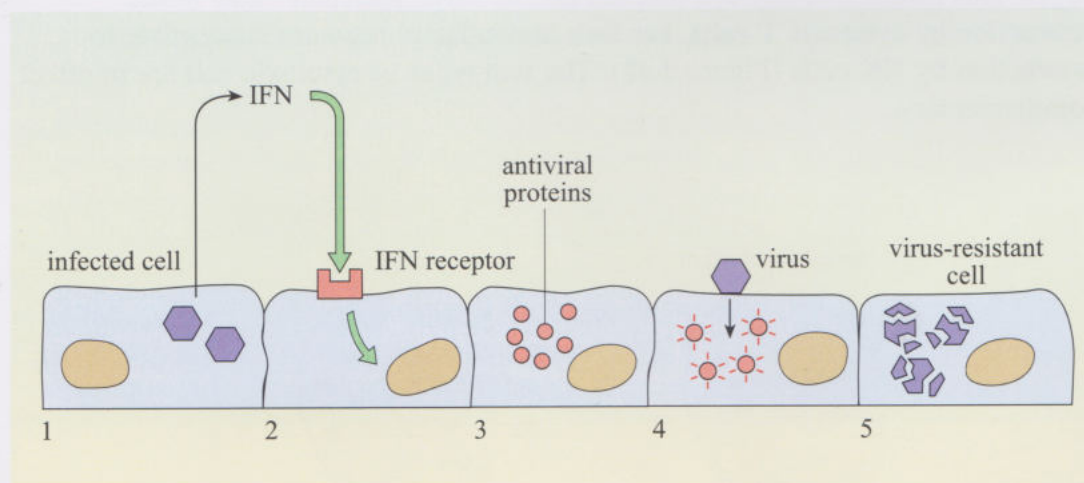


FIGURE 2.47 Interferons (IFN) are released by infected cells (1) and bind to interferon receptors, which are widely distributed on different cell types (2). This causes the synthesis of antiviral proteins (3) which are normally inactive. If the cell becomes infected with a virus, molecules such as double stranded RNA produced by the virus activate the anti-viral proteins (4), putting the cell into a resistant state so that viral replication is inhibited (5).

- ☐ Of what value is it for a cell to shut down its protein synthesis?
- ☒ Ordinarily, it would be very damaging for a cell to stop protein synthesis, but infection is a special case. By preventing protein synthesis, the cell stops the infecting virus from synthesising its coat proteins and any enzymes required for its replication. Consequently the spread of the virus is slowed. It is bad news for the infected cell, but beneficial for the host.

You have seen that $\text{IFN}\gamma$ has many functions, including the induction of MHC molecules. Both $\text{IFN}\alpha$ and $\text{IFN}\beta$ can also induce MHC molecules, although they do not perform the other functions of $\text{IFN}\gamma$ such as macrophage activation or induction of endothelial adhesion molecules. On the other hand, $\text{IFN}\alpha$ and $\text{IFN}\beta$ are more effective at the anti-viral functions described above, and are generally produced earlier in an infection, before the immune response with $\text{IFN}\gamma$ production develops fully.

2.13.2 Cytotoxic T cells and NK cells

Recognition by Tc cells and NK cells

Both cytotoxic T cells and large granular lymphocytes (NK cells) can kill cells which have become infected with virus. However, they recognize their targets in completely different ways. When considering antigen presentation, it was clear that Tc cells recognize antigen fragments presented by MHC class I molecules, which are present on virtually all cells.

Many viruses, when they have infected a cell, down-regulate the synthesis of its MHC class I molecules. This is very logical. If a cell lacks MHC class I, it no longer presents its internal molecules and so cannot be recognized by the Tc cells. For example, human immunodeficiency virus, HIV, destabilizes newly synthesized class I molecules. Another example is adenoviruses, which cause the class I molecules to be retained in the endoplasmic reticulum so they are not expressed at the cell surface.

It is here that the NK cell comes into play. NK cells have receptors which recognize MHC class I molecules. They are called killer inhibitory receptors (KIRs) because, if the NK cell recognizes an MHC class I molecule, it is inhibited from killing the target. This means that any cell that has lost its MHC molecules may evade destruction by cytotoxic T cells, but then immediately becomes susceptible to destruction by NK cells (Figure 2.48). The two types of cytotoxic cell are in effect complementary.

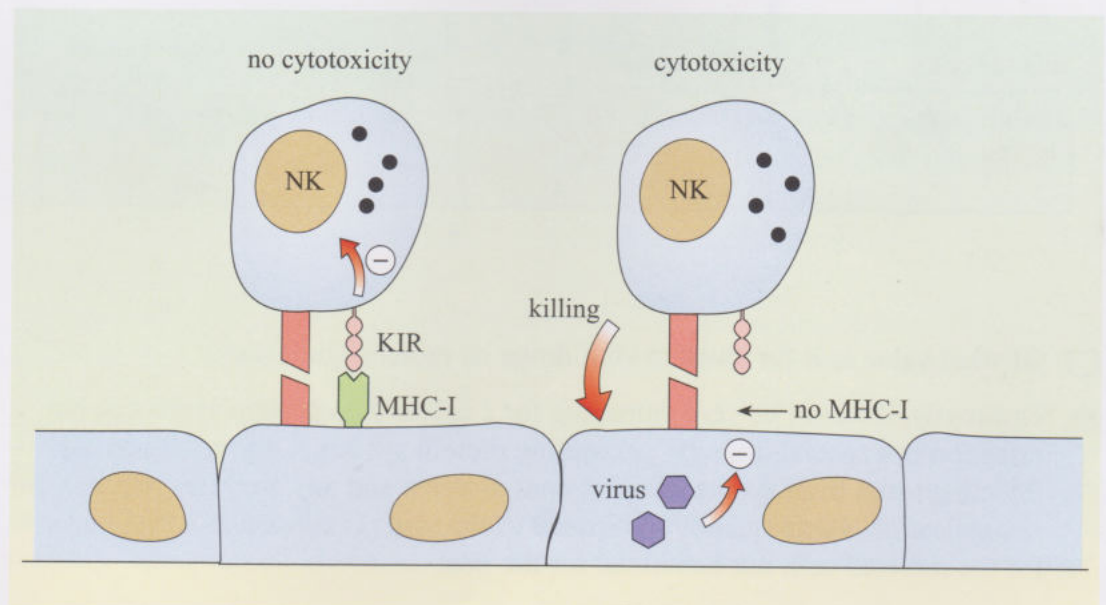


FIGURE 2.48
Natural killer cells interact with cells of the body via a number of receptors, including killer inhibitory receptors (KIR). If a cell of the body expresses class-I MHC molecules these receptors engage the class I molecule and cytotoxicity is inhibited (left). Many viruses down-regulate the expression of MHC class I molecules. However the absence of these molecules means that the NK cell is no longer inhibited from killing the target (right).

NK cells do in fact have at least two different types of receptor for MHC class I molecules, and they are quite distinct from either T cell receptors or antibodies. In addition, the NK cell has to positively identify its target. It can use a number of receptors for this function, but it can also enlist the help of antibodies as adapter molecules. NK cells have a receptor for IgG (FcγRIII), which allows them to bind to antibodies that have attached to viral molecules on the surface of the infected cell. This action is called **antibody-dependent cell-mediated cytotoxicity (ADCC)**; see Figure 2.49).

- In what circumstances might there be viral antigens on the surface of an infected cell, which could be recognized by antibodies?
- Enveloped viruses insert viral antigens into the plasma membrane of an infected cell. These molecules can be recognized by antibodies, but remember that antibodies cannot recognize antigenic peptides presented by MHC molecules.

ADCC was first identified many years ago and large granular lymphocytes were seen to be most effective at this function. Cells which carry out ADCC were called killer (K) cells. As understanding of NK cell function became clear, ADCC (K cell activity) has come to be seen as one particular way in which NK cells recognize their targets.

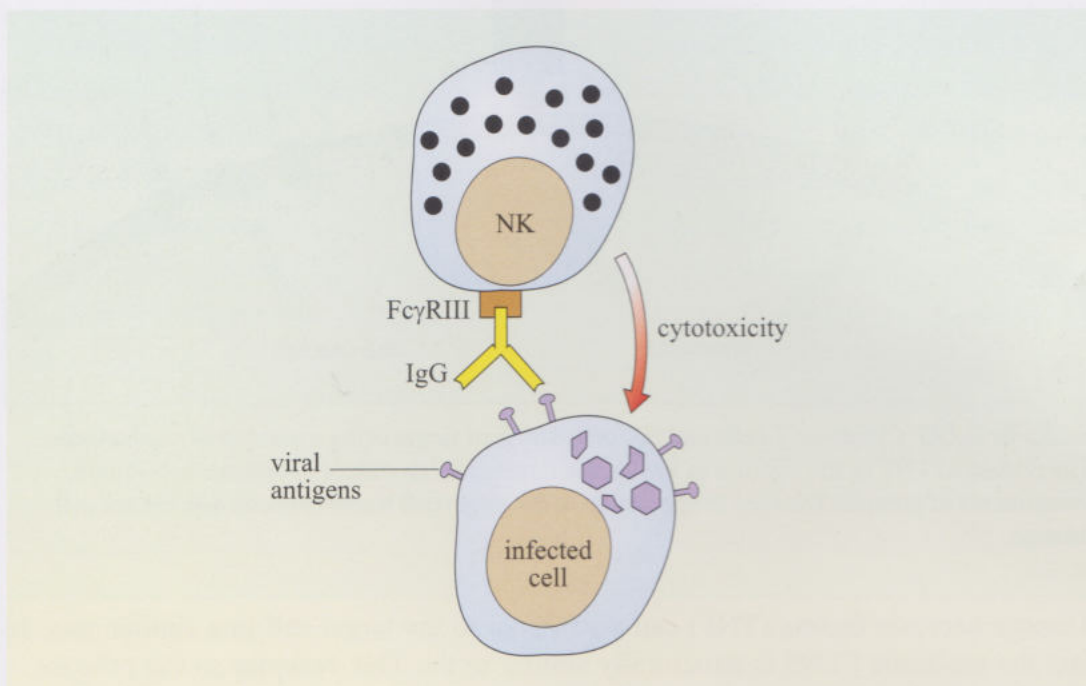


FIGURE 2.49 NK cells can recognize their targets using antibody bound to antigens on the infected cell surface, via their Fc receptors. This function, using antibody to recognize the target, is called killer (K) cell activity.

Mechanisms of cytotoxicity

Having recognized their targets, Tc cells and NK cells have several mechanisms by which they can kill the target cell. Some of these require direct cell-cell interactions, while others involve cytokines or secreted molecules (Figure 2.50). One interaction involves a molecule called Fas (CD95) which is present on the surface of many cells of the body. If this molecule is cross-linked by binding to its ligand on the Tc cell (CD95L) it initiates a train of intracellular signals which lead to cell death by apoptosis. In effect, the cytotoxic cell instructs the infected target cell to commit suicide.

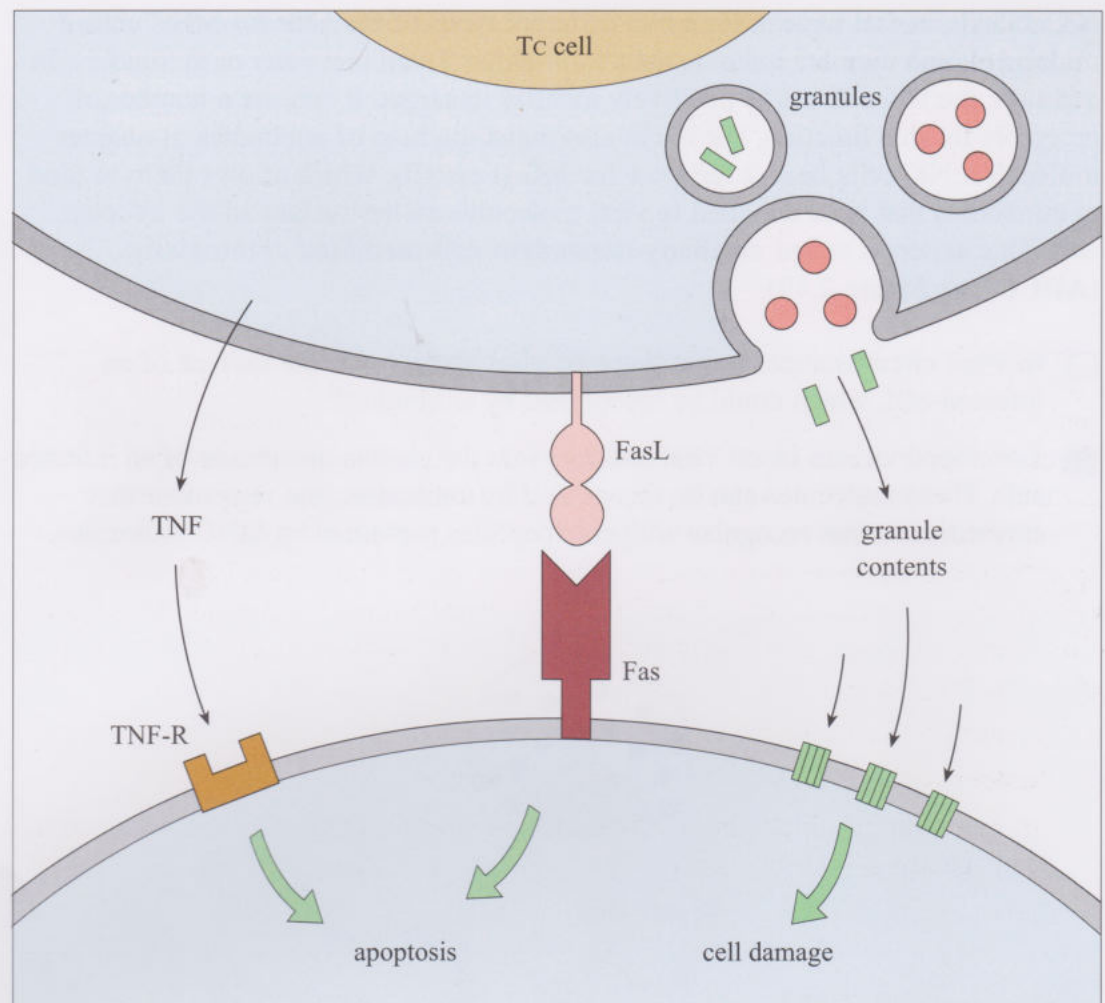


FIGURE 2.50 Cytotoxic T cells and NK cells kill their target using a number of mechanisms. The release of TNF or the ligation of Fas on the target cell can induce apoptosis. In addition, components of granules released in apposition to the target cell membrane can also induce cell damage.

Tumour necrosis factors (TNF) can also signal to the target cell in a similar way. In fact the molecule CD95 is structurally similar to the TNF receptor so the process for passing the suicide signal is similar. In each case it involves trimerization of the receptor, i.e. three receptors are aggregated at the cell surface by the cytokine or the ligand. The receptors have intracellular domains that recruit molecules involved in the killing pathway. The cascade of intracellular signals leads to the activation of a group of enzymes called caspases, which are involved in initiating cell death. Caspases are normally present in the cell, but in an inactive form. (Note that although TNF was first identified for its function in killing tumours, its role in killing infected cells and controlling inflammation is much more important). A cell that dies by apoptosis condenses, its membrane coalesces into small fragments (blebs), its DNA breaks down and it eventually disintegrates. Macrophages, as usual, clear up the debris.

In addition to these signalling systems, Tc cells and NK cells have killing systems that use the contents of their granules (Figure 2.50). When a cytotoxic T cell engages a target it moves its granules towards the junction between the two cells. The granules then fuse with the plasma membrane, releasing the granule contents directly against the membrane of the target. The granule contents include a molecule called perforin that punches holes in the membrane of the infected cell.

- ☐ Can you recall any other immunological system that is able to punch holes in the plasma membranes of cells?
- ☒ The lytic pathway of complement (Figure 2.10) does exactly this when it assembles a membrane attack complex on a target cell following complement activation via the classical or alternative pathways. The final component of the lytic pathway of complement is C9 which is structurally homologous to perforin.

Also contained within the granules are a series of granule-associated enzymes, or 'granzymes'. Some of the granzymes are able to link in to the intracellular signalling pathways that lead to the activation of caspases. It is thought therefore that the holes that are produced by perforin allow granzymes to enter the target cell and initiate the process of apoptosis (Figure 2.51). Other granzymes appear to have a direct action in breaking down cellular proteins. In addition to allowing granzymes to enter the target cell, perforin pores allow ions to leak out of the cell which disregulates its normal function and it allows water to move in, leading to osmotic disruption. So the cytotoxic cells have at least three ways in which they can kill cells that they recognize as infected:

- ligation of Fas initiates apoptosis;
- tumour necrosis factors also signals apoptosis;
- granule proteins, including perforin and granzymes, cause physical and osmotic damage and induce apoptosis.

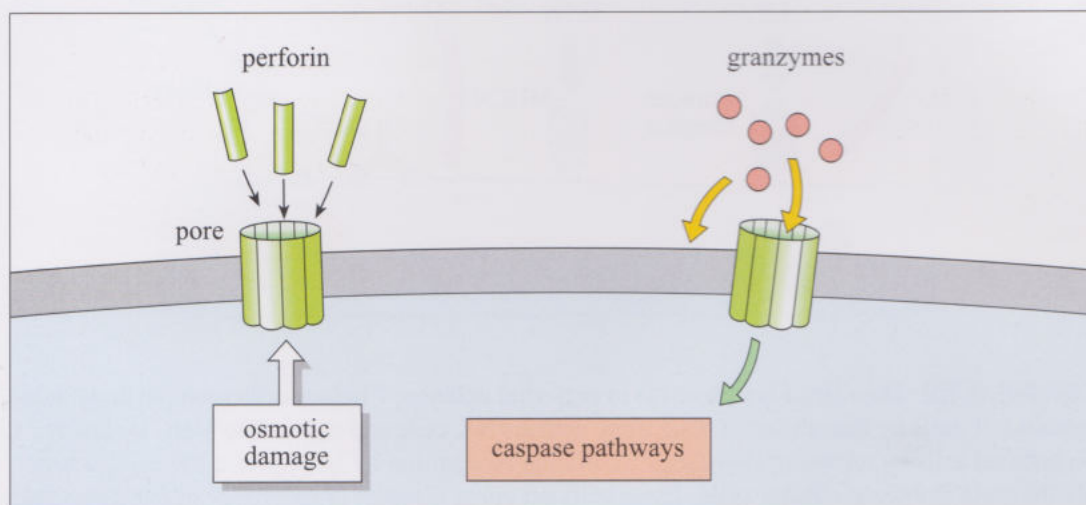


FIGURE 2.51 Granule-associated killing mechanisms. Perforin polymerizes in the membrane of the target cell, producing pores which allow the flow of water, ions and larger molecules into and out of the target cell. Granule-associated enzymes (granzymes) are enzymes that can either directly damage the target, or may pass into the target cell to engage the caspase pathways that lead to apoptosis.

The relative importance of each mechanism will depend partly on the type of cell involved. You will recall that NK cells are large granular lymphocytes, so they are particularly well equipped with the granule-associated killing systems. Cytotoxic T cells will use the Fas system, granules and cytokines.

2.13.3 The role of CD4⁺ T cells

Immunologists often use the abbreviation CD4⁺, CD16⁺, etc., to indicate that a cell has those markers. To this point we have used CD8 as the definitive marker of Tc cells but we want to discuss some exceptions – in this case a CD4⁺ Tc cell.

In discussing anti-viral defences, we have not yet discussed the role of CD4⁺ T cells. Certainly these cells do contribute to the process, since cytokines such as IL-2, which promote T cell division, act on CD8⁺ Tc cells, as well as CD4⁺ T cells. In addition, IFN γ released by T helper cells enhances the actions of NK cells. Moreover, as it increases the expression of MHC molecules, it makes cells of the body more readily recognizable by Tc cells and NK cells. However, in special circumstances CD4⁺ T cells can also act as cytotoxic cells themselves. Amongst the cytokines they produce, CD4⁺ T cells release one that is a variant of tumour necrosis factor called lymphotoxin (LT) or TNF β . This mediates killing in much the same way as TNF α , by ligating a TNF receptor on the infected cell. Notwithstanding the possible cytotoxic role of CD4⁺ T cells, it appears that the CD8⁺ Tc cells and the NK cells are pre-eminent in cell killing, while CD4⁺ T cells contribute to the development proliferation and actions of the cytotoxic cells (Figure 2.52).

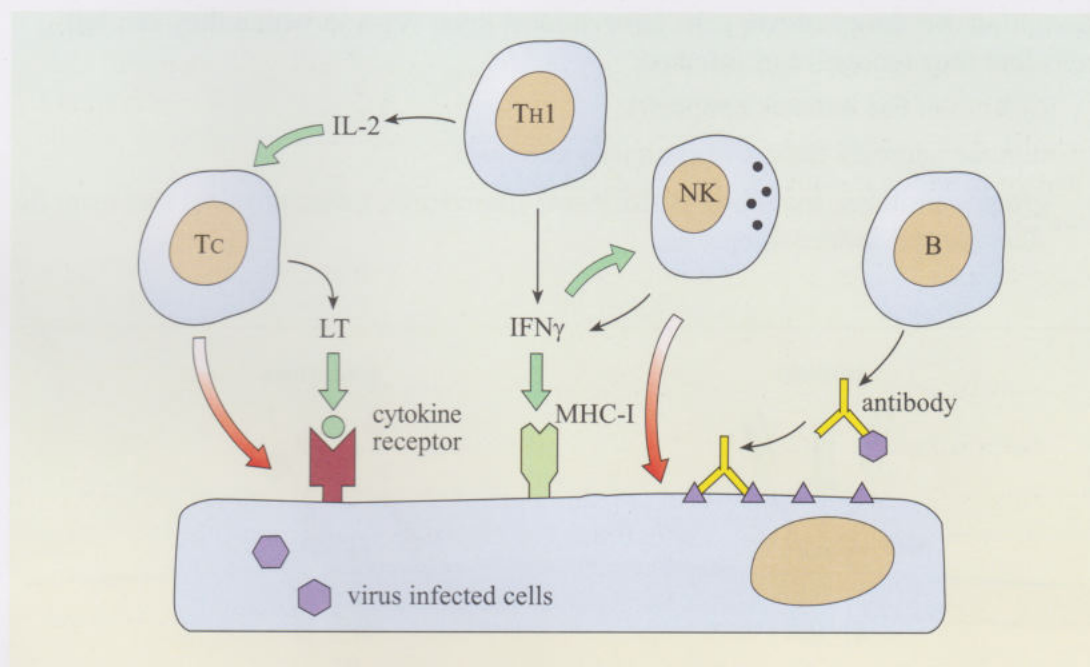


FIGURE 2.52 The role of lymphocytes in anti-viral defence. T helper cells promote division of cytotoxic T cells by the release of IL-2. They activate NK cells and upregulate MHC molecules on infected cells by release of IFN γ , thus facilitating recognition by Tc cells and NK cells, which are the main cytotoxic effector cells. These cells act either directly, or by release of cytokines such as lymphotoxin (LT). B cells produce antiviral antibodies that can aid recognition of infected cells by NK cells as well as limiting the spread of free virus.

2.13.4 Susceptibility of cells to cytotoxic damage

The susceptibility of infected cells to the killing mechanisms described is not uniform. Cells vary in their ability to withstand osmotic damage, and in their resistance to cytotoxicity signalled via Fas and cytokines. In particular, neurons and stem cells normally express low levels of MHC molecules, which theoretically would make them susceptible to attack by NK cells. Despite this, it is unusual for cytotoxic cells to kill these types of cell. They appear to have special mechanisms

that partly protect them. This is generally desirable; stem cells and neurons must last a lifetime and destroying them is not a good idea. An example of the devastation caused by an immune attack on these cells is seen in rare individuals who develop an encephalitis (severe inflammation of the brain) following infection with *herpes simplex*. The immune-mediated attack destroys neurons in the hippocampus, the area of the brain responsible for laying down new memories. The unfortunate individuals lose the ability to make new memories and therefore live in a sort of permanent present. The immune system has destroyed the infected cells, but it is a Pyrrhic victory. The preferred option is to tolerate the infected cells, but then they act as a permanent source of infection throughout life – it is a difficult balance that the immune system must maintain.

2.13.5 Phasing of anti-viral defences

At this stage we should review the various antiviral defences which are available and relate this to the time at which they are most effective. Interferons are important in the earliest phases of infection when it is important to slow the infection. At this stage NK cells are also available in significant numbers, and can kill cells which attempt to evade recognition by down-regulating MHC class I molecules. As the immune response develops, virus-specific Tc cells and antigen-specific B cells are produced in increasing numbers. The Tc cells can eliminate virus-infected cells directly, while antibodies produced by the B cells have many functions:

- preventing virus from binding to receptors on cells they might otherwise infect;
- targeting NK cells to kill infected cells;
- activating the complement classical and lytic pathways;
- opsonising free virus for uptake by phagocytes.

After the infection has been cleared, antibodies persist in the blood and tissue fluids for many months to prevent reinfection with the same virus (Figure 2.53).

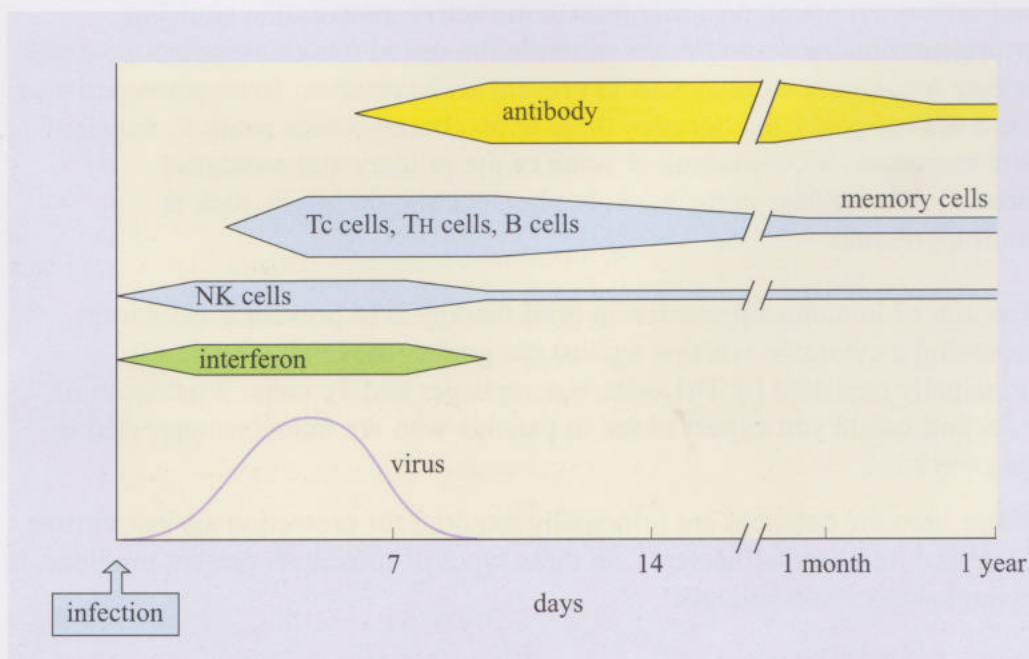


FIGURE 2.53

The immune defences operating against an acute viral infection are shown in relation to the time course of the infection. Interferon and NK cells are active during the earliest phases of infection as the virus infects cells. Specific responses mediated by lymphocytes develop a few days after infection and are involved in clearing the infection. The differentiation of memory cells is important in the development of immunity to reinfection. Antibody develops after the expansion of virus-specific B cells, by the end of the first week. Antibody levels decay over a number of months, but residual amounts may persist for a long period, as a result of reactivation of memory B cells.

2.13.6 Summary of anti-viral defences (CD1)



You should now go to the CD1 topic entitled *Anti-viral defences*. This will allow you to consolidate your understanding of the subject. The expansion screens entitled 'Immune defences against viruses', 'Defence against generalized virus infection' and 'Cell-mediated cytotoxicity' provide summaries of the major anti-viral defences. You should also see the screens entitled 'viral antigens' and 'NK cell receptors' as they provide some additional material that is not included in the text above.

Be sure to view the time lapse video sequence of a 'cytotoxic T cell killing a virus-infected cell'. It is very striking.

After you have worked through the topic and expansion screens you should be able to answer Questions 1–9 in the self-assessment section.

You should spend about one hour on this animation and associated questions.

2.14 Immunosuppression

We have now reviewed most of the principal mechanisms that the immune system deploys to protect the body against infection. You have seen that different mechanisms are appropriate for each type of pathogen, but what if the immune system breaks down or cannot cope with an infection? In this case pathogens can gain the upper hand, and the body becomes overwhelmed. Sometimes normally harmless commensal organisms act as dangerous pathogens.

We can distinguish two different ways in which the immune system can fail, either because there is some underlying immunodeficiency or because the system is suppressed. An **immunodeficiency** occurs when some element of the immune defences is lacking. This may be a *primary* deficiency, caused by a genetic defect, or a *secondary* immunodeficiency caused by an external agent such as the AIDS virus, HIV, or by malnutrition. We use the term **immunosuppression** to describe reduced immune function resulting from some active intervention aimed at preventing an immune response, for example the use of immunosuppressive drugs to alleviate autoimmune diseases or to prevent graft rejection. In common parlance, there is a deal of confusion between these terms, because both result in impaired immune responses. We shall look at some of the primary and secondary immunodeficiencies later in the book, but here we should briefly look at immunosuppression.

- ☐ The aim of immunosuppression in graft therapy is to prevent T cells from mounting a cytotoxic reaction against the graft cells. Graft rejection is principally mediated by T_H1 cells, macrophages and T_C cells. What types of infection would you expect to see in patients who are immunosuppressed in this way?
- ☒ These immune defences are principally required for protection against viruses, fungi and intracellular bacteria. So these types of infections present problems in immunosuppressed patients.

An example of this problem occurs with cytomegalovirus (CMV) in transplant recipients who are immunosuppressed to prevent them from rejecting their graft. Cytomegalovirus is a herpes virus that very occasionally produces a disease resembling glandular fever. It is however the most common cause of congenital viral infection. Many people are permanently latently infected with cytomegalovirus, which the immune system keeps in check. If the graft has cytomegalovirus in it, or if they have a latent infection themselves, the immunosuppression can allow the virus to escape from control. Before pre-screening was introduced, up to 35% of transplant patients could develop CMV infection, with gastro-enteritis, pneumonia, and other organs affected. The infection could be fatal, but more often the immunosuppressive drugs were suspended, the infection was controlled, but the graft was rejected. Nowadays, anti-viral drugs are used to control CMV in the period immediately after transplantation. Nevertheless, reactivation of infectious agents, including viruses, is still a major problem in immunosuppressed patients (Figure 2.54).



FIGURE 2.54
Oral lesions of herpes simplex in a child who received a graft 14 days previously and has undergone immunosuppressive therapy. In this circumstance the immune system is unable to control the infection as normal.

Summary of Sections 2.9–2.14

- 1 MHC molecules present antigen fragments to T cells. Antigen from within cells, processed by the internal pathway, is presented by MHC class I molecules, and antigens that have been endocytosed (external pathway) are presented by class II molecules.
- 2 In order to be activated, T cells require a signal of antigen presented on an MHC molecule and costimulation. Infectious agents may promote antigen presentation by enhancing costimulation.
- 3 T-independent antigens can stimulate B cells directly to secrete antibody, but T dependent antigens require the B cell to receive help from T cells.
- 4 Individual B cells start by producing IgM, but can later switch to produce other antibody classes. This change is reflected in the antibodies seen in the blood as an immune response develops. Antibody affinity rises during an immune response as IgA, IgG and IgE are produced.
- 5 Helper T cells fall into two categories: T_H1 cells release cytokines which promote the activation of macrophages; T_H2 cells release cytokines which promote antibody production. Helper T cells interact with a restricted group of antigen presenting cells, which include B cells, macrophages and dendritic cells.
- 6 Whether an individual makes a T_H1 -type response or a T_H2 type of response to a pathogen, depends on their genetic make-up and on how the antigens are first presented to the lymphocytes. T_H1 cells tend to turn off T_H2 cells and vice versa.
- 7 Cytotoxic T cells recognise antigen presented by MHC class I molecules on the surface of virally-infected cells. Any nucleated cell can present antigen to a cytotoxic T cell. A cytotoxic T cell can then signal the infected cell to die by

apoptosis. NK cells recognise and react against infected cells that have down-regulated their MHC class I molecules.

- 8 Cytotoxic T cells and NK cells are important in clearing virally-infected cells. Antibodies can limit viral spread in the extracellular spaces, and interferons are important in limiting the ability of virus to replicate within cells.
- 9 Immunological memory is a key component of long-lasting immunity to an infectious agent. The basis of memory is the proliferation of lymphocytes during the primary immune response. Some of these cells become very long-lived memory cells, which have differentiated, so that they are more readily activated following a second encounter with the same antigen.
- 10 In individuals who are immunosuppressed, organisms that are normally harmless may become pathogenic.

Learning outcomes for Chapter 2

- 2.1 Define and use, or recognise definitions and applications of, each of the terms printed in **bold** in the text.
- 2.2 Describe the structure and organisation of the different antibody classes and their role in immune responses.
- 2.3 Give descriptions of the diverse range of immune responses which the body deploys to deal with infectious agents and explain why different immune responses are appropriate defences against particular pathogens.
- 2.4 Explain antibody binding mechanisms with reference to molecular structure, and relate this to the definitions of affinity, avidity and cross-reactivity.
- 2.5 Describe the mechanisms employed in the immune response against influenza and why the influenza virus is so successful at surviving.
- 2.6 Describe how the complement system is activated and functions in the control of inflammation and immune defence.
- 2.7 Explain what constitutes an effective or appropriate immune response, giving a number of different examples.
- 2.8 Describe the mechanisms by which phagocytes kill pathogens and break down dead pathogens and immune complexes. Relate these processes to antigen presentation.
- 2.9 Explain the importance of mononuclear phagocytes and macrophages in defence against bacterial infections, with particular reference to typhoid, TB and leprosy.
- 2.10 Describe the MHC and the importance of antigen processing and antigen presentation for both class I and class II pathway molecules.
- 2.11 Explain the requirements for T cell activation, including costimulation.
- 2.12 Explain how class-switching in the immune response can be related to class-switching by individual B cells and class-switching within the genome of the individual cell. Relate this to the role of T cells and dendritic cells.

- 2.13 Explain how cytokines can modulate the balance between a T_H1 -type of immune response and a T_H2 response.
- 2.14 List the types of immune defence which can protect against viral infections. Relate this to the time of action, and the cellular location of the virus.
- 2.15 Relate different types of immunodeficiency or immunosuppression to the types of infection seen in those conditions.
- 2.16 Explain the cellular basis of immunological memory.

It is the policy of the Association to publish only original research articles of broad interest to the medical profession. The Journal is not a place for the publication of routine clinical reports, case reports, or reviews of the literature. The Journal is also not a place for the publication of articles that are purely theoretical or that are of only local interest. The Journal is a place for the publication of articles that are of broad interest to the medical profession and that are of high scientific quality. The Journal is a place for the publication of articles that are of broad interest to the medical profession and that are of high scientific quality.

3 HIV AND AIDS: A CASE STUDY

You have now looked at the ways in which the body responds to viral infections. We are now going to consider human immunodeficiency virus (HIV) infection and the consequence of infection, acquired immunodeficiency syndrome (AIDS). Not only is this a serious and globally important disease, but it also provides an example of an infection where the virus selectively targets cells of the immune system. This leads to immunodeficiency so that secondary infections with other pathogens develop.

The case study uses the 'Topics in International Health Series' CD-ROM entitled *HIV/AIDS*. This disc contains 11 interactive tutorials, which would take 7–8 hours to go through exhaustively. You should aim to spend about three hours on this case study, doing just four tutorials as indicated below. The remaining material on the CD-ROM may be used for project work, or for general interest.

Work through:

- Biology of HIV
- Epidemiology
- Two more tutorials selected according to your interest.

If you are interested in the social, political and economic dimensions of HIV/AIDS, study the material in U205, Book 8, Chapter 6, which can be found on the S320 *Reference CD*.



3.1 Human Immunodeficiency Virus mini-lecture (CD2)

You should now view the mini-lecture entitled *Human Immunodeficiency Virus* on *Immunology Interactive CD2*. This lecture summarizes some of the material you have already encountered in the case study and it develops themes concerned with the immunology of AIDS, and the immune responses that are directed against the virus.

Spend about 45 minutes studying the lecture.

After you have listened to the lecture, you should be able to answer the following questions (the answers are given at the end of the book):



Question 3.1

How does the virus enter cells of the immune system?

Question 3.2

What determines whether a virus can infect T cells or macrophages?

Question 3.3

Why do the genes of some individuals protect them against infection and disease progression?

Question 3.4

What types of immune response develop against HIV?

Question 3.5

What characteristic of the virus enables it to escape from control by the immune response?

Question 3.6

What types of infection and disease are seen in individuals who have developed AIDS?

Question 3.7

Why do they develop just this group of infections and not others?

4 SECONDARY CONSEQUENCES OF INFECTION

The case study on AIDS provides a good example of a disease in which the serious pathology is not caused by the primary infection with HIV, but is due to the opportunistic infections that develop as a consequence. There are several other ways in which an infection can lead to secondary disease, including:

- immunodeficiency;
- hypersensitivity;
- autoimmunity;
- oncogenesis – the development of tumours.

You have already seen the consequences of immunodeficiency, not just in AIDS but also in the examples of genetic deficiencies which lead to increased susceptibility to infection. **Hypersensitivity** is defined as an immune response, where the reaction is out of proportion to the damage caused by the antigen, and does more harm than good. **Autoimmunity** covers a group of diseases, in which the immune system reacts against the body's own cells. This section considers mechanisms by which infection can lead to secondary disease.

4.1 Tumour development

We will first look at tumour development. If infection leads to the production of tumours, then one would expect to see an increased incidence of tumours in people with immunodeficiency, since they are more susceptible to infection. It is true that some tumours are associated with immunodeficiency (e.g. Kaposi's sarcoma) but the effect is rather selective. Some tumours are increased in immunodeficient people, but most of the common tumours are not. The key to the problem is in identifying what causes the particular type of tumour.

Most tumours are caused by mutations in the cellular DNA due to chemical or physical causes, for example carcinogens or radiation. Cells protect themselves against mutations by continuously repairing their DNA and this is our primary protection against tumours. The immune system is not usually of much value in protecting against such tumours. This is because the mutations caused in the proteins are generally very subtle and may not be recognized by the immune system. Moreover, tumours develop insidiously and so may be tolerated by the body even if the immune system recognizes them as non-self. However, a few tumours are associated with viral or bacterial infections and because the immune system protects against infection, such tumours are often more prevalent in immunodeficient people. Examples of tumours that are associated with infection are given in Table 4.1.

TABLE 4.1 Tumours associated with infectious diseases.

Infection	Tumour	Notes
herpes virus type VIII	Kaposi's sarcoma	in HIV/AIDS patients
papilloma viruses	cervical cancer bladder cancer cutaneous cancers	
HTLV-1 virus	T cell leukaemia	
Epstein Barr virus	Burkitt's lymphoma nasopharyngeal carcinoma	in central Africa in SouthEast Asia
hepatitis-B and C viruses	liver carcinoma	

- ☐ Are there any factors that are common to the viruses which can induce tumours?
- ☒ They all establish chronic infections, but beyond that they are quite diverse as they belong to different groups.

Because retroviruses break and rejoin DNA when provirus becomes incorporated into the cell's DNA, it is easy to see how viruses such as HIV or HTLV-1 (Human T cell leukaemia virus) could disrupt the expression of genes which control the cell cycle. But even in the case of HTLV-1, only about 2% of infected individuals develop leukaemia, so the virus infection alone is insufficient to cause that disease.

It is less easy to understand why herpes viruses are associated with tumours. Since these viruses are very widespread and the incidence of the tumours they associate with is low, it implies that the virus is only one of many factors contributing to tumour development. However, herpes viruses are capable of profoundly affecting cellular metabolism as they establish latent infections. They carry genes that interfere with the pathways of apoptosis, therefore they can promote cell survival. (We shall return to this subject, when we look at how herpes viruses evade immune responses.)

Epstein Barr virus (EBV) is a herpes virus that is the causative agent of glandular fever in most areas of the world. Glandular fever is an infection that causes sore throat, fatigue, malaise and swollen lymph nodes, with symptoms lasting for a few weeks. However, in areas of Africa it is associated with a condition called Burkitt's lymphoma, and in the Far East it is associated with a nasopharyngeal carcinoma (Figure 4.1). In all cases the affected cells are B cells. Epstein Barr virus infects B cells and causes them to proliferate. In normal individuals, the proliferating cells are controlled by cytotoxic T cells but in rare individuals who have a genetic defect (X-linked lymphoproliferative syndrome), the infected B cells divide unchecked and the condition is often fatal. Patients with Burkitt's lymphoma are usually children who have swollen lymph nodes in the jaw and orbital cavity, while those with nasopharyngeal carcinoma, are more generally older men. In all cases the B cells produce a lymphoma, but the diseases affect different groups of individuals in particular geographic locations. This implies that the virus is necessary but not sufficient to develop the lymphoma, and that genetic and environmental factors also contribute.

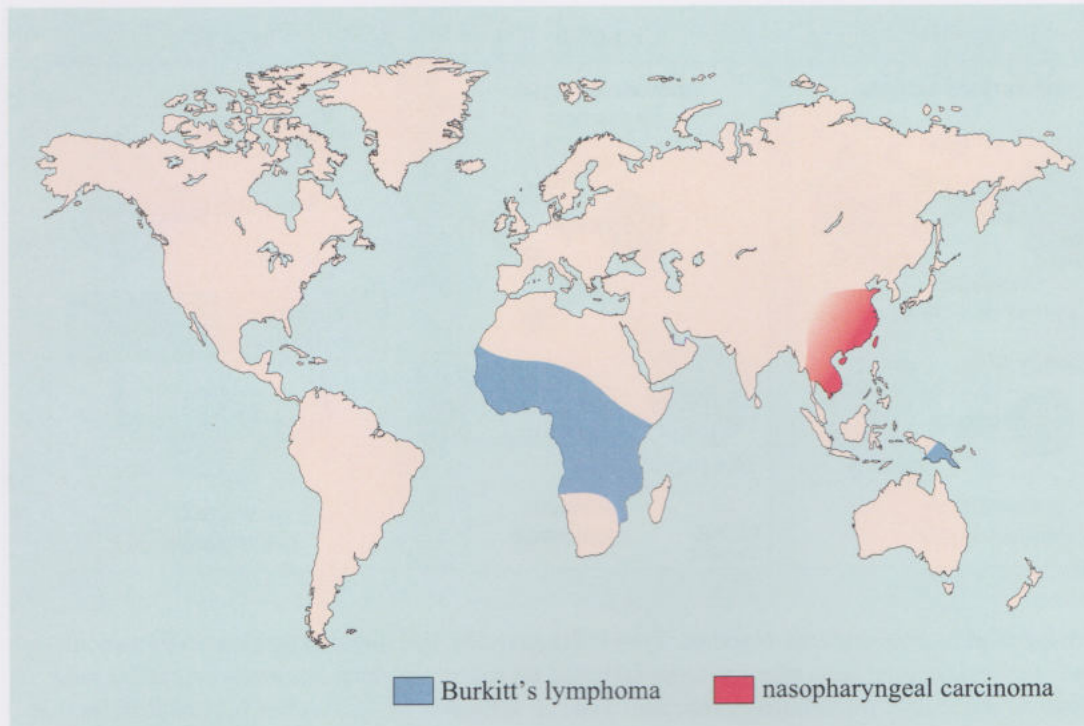


FIGURE 4.1 The geographical distribution of tumours associated with Epstein Barr virus infection. Burkitt's lymphoma is endemic in Central Africa and Guinea, while nasopharyngeal carcinoma occurs predominately in Southern China, but is also reported in Innuits. Sporadic cases can occur in any country.

A lymphoma is a type of carcinoma. Nasopharyngeal carcinoma is the term in general use, but it is a lymphoma.

The hepatitis viruses present another type of infection. They are associated with liver cancer. Again, the incidence of this tumour is low, although the viruses are very common. In this case, it is thought that chronic liver damage and regeneration means that the liver cells will be primed to respond to growth factors. The receptors and genes that allow cells to respond to growth factors are often expressed on tumours. So their chronic induction as a result of the infection, bypasses one of the steps which the cells need to transform into a tumour.

4.2 Hypersensitivity

Hypersensitivity is another area where infection can lead to disease. To understand the CD2 mini-lecture on this subject, which refers to the four types of hypersensitivity, some background material is needed.

When considering hypersensitivity, Gell and Coombs classified hypersensitivity according to four different mechanisms. Although there is some overlap between the mechanisms, because each one does not necessarily occur in isolation, this classification is still current and useful (Figure 4.2).

Type I hypersensitivity reactions develop very quickly after contact with an antigen. They occur in individuals who have a high level of IgE to the antigen. Mast cells are then triggered to release their inflammatory mediators, including histamine, prostaglandins and leukotrienes. If the reaction is localized, it will cause local inflammation. A typical example of this is hayfever, where antigens from pollen trigger inflammation in the nose, eyes and respiratory tract. Much more dangerous are generalized type-I reactions, when antigen enters the blood stream: there is a widespread release of the mediators that causes dilation of arterioles, a loss of blood

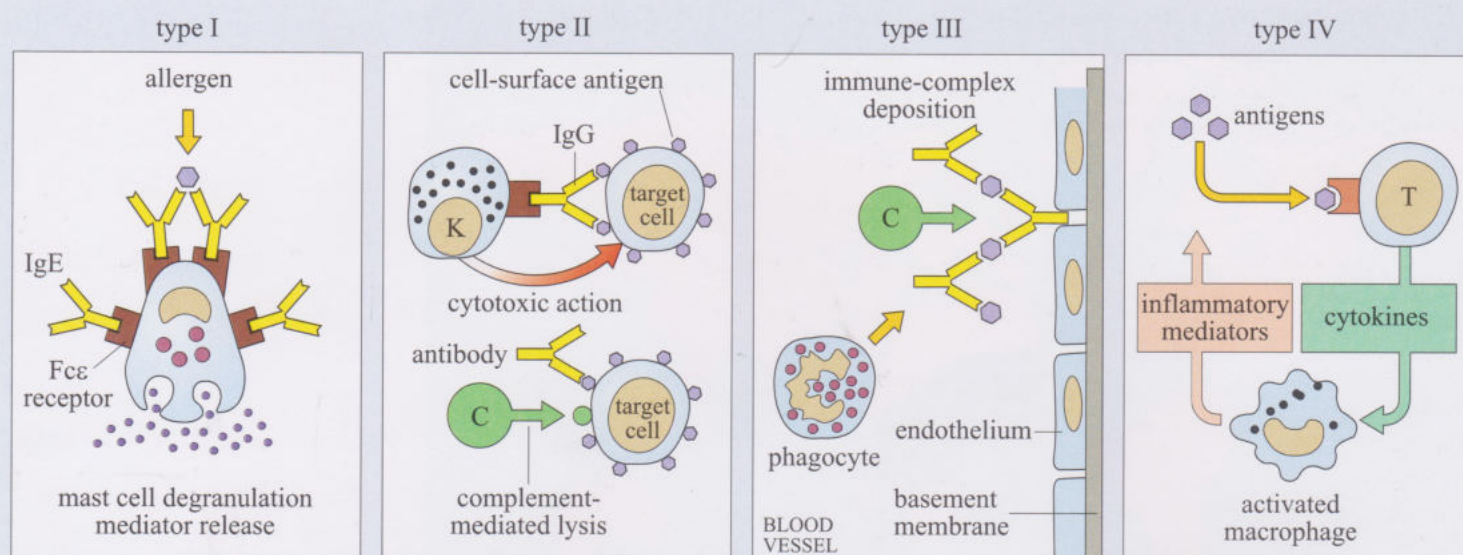


FIGURE 4.2 An overview of the four main types of hypersensitivity reaction. Type I: is caused by IgE directed against an innocuous antigen (an allergen). Mast cells bind IgE on their Fc receptors. If an allergen cross-links the receptors it causes degranulation of the mast cells with the release of mediators that provoke an immediate inflammatory reaction. Type II: is caused by IgG and/or IgM antibodies that recognize cells or components of tissues. The antibodies bind to the cell or tissue and cause complement activation and lysis, or can direct large granular lymphocytes (K cells) or macrophages onto the target. Type III: is caused by antigen-antibody immune complexes produced in large quantities. The complexes cannot be cleared normally by phagocytes and circulate, becoming deposited in blood vessels of organs such as the kidney, where they provoke local inflammatory reactions and damage. The damage develops over a number of hours or days. Type IV: is caused by sensitized T cells, which release inflammatory cytokines, causing macrophage activation and tissue damage due to their mediators. These reactions develop over days, weeks or months.

pressure and constriction of the airways. This type of reaction is called anaphylaxis. These reactions occur most commonly following wasp or bee stings, however they can occur in infectious diseases, and the example given in the lecture concerns a worm infection.

Type II hypersensitivity reactions occur when IgG or IgM antibodies cause the damage. This kind of reaction often occurs in autoimmunity, but is not generally involved in infection.

Type III hypersensitivity reactions occur when the body has large loads of antigen to deal with. This can occur in chronic infections, such as malaria or viral hepatitis. Immune complexes are formed in large quantities – so great that it exceeds the capacity of the mononuclear phagocytes to remove them from the blood stream. The complexes often become deposited at sites of filtration, especially in the kidney, and this leads to inflammatory damage in that organ. Type III reactions also develop in a condition called ‘Farmer’s lung’. Farmers often have to work in dusty environments such as barns containing a lot of fungi. The spores from fungi are breathed into the lung in large quantities. The fungal antigens combine with antibodies in the lung to form immune complexes and these induce local inflammation and release of inflammatory cytokines which produce symptoms of asthma and sickness.

☐ Do you think Farmer’s lung is an infectious disease?

☒ It is not so really. Although it is caused by a microorganism, the fungal spores do not establish an infection in the person. They merely supply a heavy load of antigen that produces the symptoms that might be associated with an infection.

Type IV hypersensitivity reactions are mediated by T cells and mononuclear phagocytes. They usually take days to develop, and are characteristically seen in chronic infections that induce a strong cell-mediated immune response, such as tuberculosis or leprosy. The damage is mediated by cytokine-activated macrophages causing collateral damage as they attempt to control the infection.

4.3 Hypersensitivity and autoimmunity following infectious disease mini-lecture (CD2)

You should now go to the topic on CD2 entitled *Hypersensitivity and autoimmunity following infectious disease*. This gives examples of a number of infectious diseases that cause hypersensitivity reactions. In each case, relate the damaging immune reaction to the mechanisms of hypersensitivity described above.

The second part of this lecture is concerned with autoimmunity. Although there are many autoimmune diseases, only a small proportion of them are specifically associated with infectious disease. Examples given in the lecture are rheumatic fever and Guillain Barré syndrome. The key to these conditions is cross-reaction between a component of the body and a component on a pathogen, where an immune reaction against the pathogen leads to an immune reaction against the host tissue.

Spend about 75 minutes on this quite difficult material.

After listening to the lecture you should be able to answer the following questions (the answers are given at the end of the book).



Question 4.1

Give examples of some infectious agents which can lead to hypersensitivity or autoimmune disease.

Question 4.2

Describe the mechanisms of the four different types of hypersensitivity.

Question 4.3

Describe mechanisms by which the presence of an infectious agent could lead to an immune attack on host tissues.

Before proceeding, take a few minutes to think about why tumours, hypersensitivity reactions and autoimmunity only develop in a tiny proportion of the patients who get infected with a particular pathogen. The next chapter will discuss this really important question in detail.

Summary of Chapter 4

- 1 Some infections lead to secondary diseases such as tumours or hypersensitivity reactions, which are more damaging than the initiating infection. Hypersensitivity reactions can be classified according to their mechanism into types I–IV.
- 2 Some infections lead to autoimmune disease in susceptible individuals. The pathogen causes a breakdown in self-tolerance; this is most often due to cross-reactivity between the pathogen antigens and self molecules, but may also relate to a change in antigen presentation.

Learning outcomes for Chapter 4

- 4.1 Define and use, or recognise definitions and applications of, each of the terms printed in **bold** in the text.
- 4.2 Describe how tumour development associated with viral or bacterial infection occurs and how specific infections overcome the body's immune defences. Give examples of infections that may lead to the development of tumours.
- 4.3 Describe mechanisms by which infections may lead to hypersensitivity or autoimmune disease.

5 ADAPTATIONS OF THE HOST AND PATHOGEN POPULATIONS

5.1 Genetics of susceptibility to infectious disease

This section is concerned with three critical questions:

- Why are some individuals susceptible to infection with a particular pathogen, while others are resistant?
- Why is the course of a disease more serious in some people than others?
- What contribution do our genes make to susceptibility to infectious diseases?

Humans are very genetically diverse, and this diversity includes genes which determine whether a pathogen can infect an individual and how the immune system reacts against it. Such genetic variation is often referred to as **polymorphism**, meaning that individual genes occur in different forms in different individuals. In 1949, the physiologist J. B. S. Haldane suggested that infectious diseases had been the principal agent for natural selection for at least 5000 years, and that they were responsible for maintaining many of the polymorphisms seen in the human genome. His idea is based on the observation that infectious disease has had a major effect on the reproductive fitness of individuals as well as on childhood mortality.

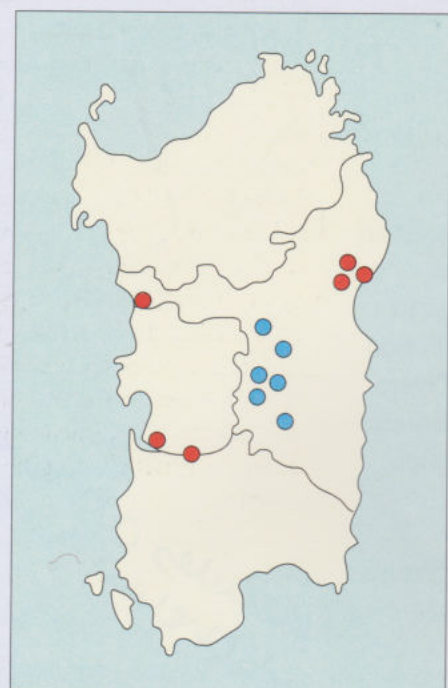
- ☐ Think back to Chapter 3 on HIV infection. Can you identify a gene that displays polymorphism, and where one variant is protective against HIV infection?
- ☒ The gene for the chemokine receptor CCR5 is polymorphic. Individuals heterozygous for the CCR5 Δ 32 mutation are partly protected from disease progression, and those who are homozygous for the variant are highly protected from infection and disease progression.

In a world where HIV infection is prevalent there is clearly some advantage to having this mutation, whereas before HIV developed it had no identifiable advantage. So the selective pressure to maintain genes in a population will vary depending on what diseases are prevalent at a particular time. Taking this idea further, we can see that the selective pressures caused by pathogens in different areas of the world vary. For example, a gene which promotes resistance to malaria would be of little selective advantage in Iceland, but very advantageous in equatorial Africa. It means that any genes that are selected because of their contribution to disease resistance will vary across the globe, in relation to the locally-prevalent infectious diseases. This proposition can be examined directly. It predicts that a polymorphism that confers resistance to a disease will be more common in areas or populations where that disease is endemic.

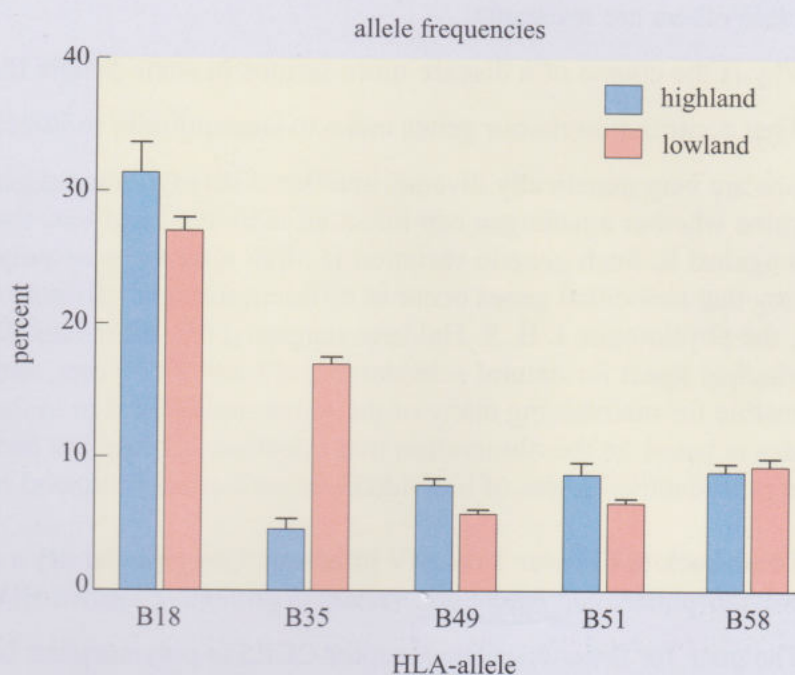
For example, one variant of an MHC class I molecule (HLA-B35) is associated with resistance to malaria. The distribution of this variant was investigated in Sardinia, an island where malaria occurred until 1948, although the disease was confined to lowland areas. The incidence of the HLA-B35 variant was investigated in both highland and lowland villages and the variant was much more common in the

lowland villages (Figure 5.1). This strongly suggests that malaria acted as an agent for selection of the resistance gene in lowland areas.

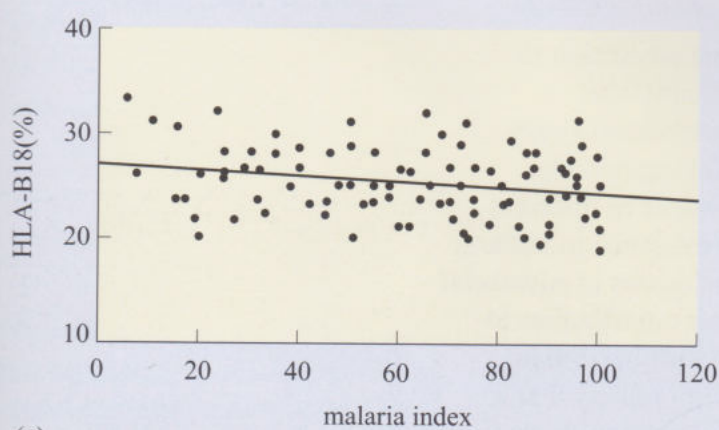
Even within a localized population the selective pressures acting on individual genes will vary depending on other genes and the behaviour of individuals. For example, a gene which protects against syphilis, hepatitis-B or HIV, would confer more advantage in a population where multiple sex partners is the norm. It might appear that the complex interactions between sets of genes, environmental factors and complex behaviour would make this subject impossibly difficult to study. However



(a)



(b)



(c)

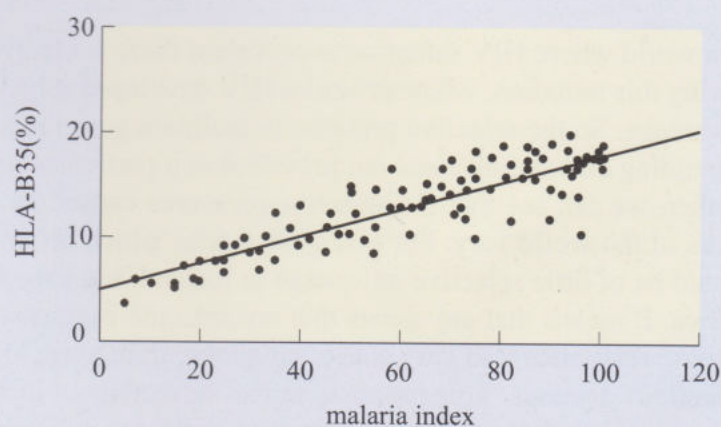


FIGURE 5.1 The frequency of selected HLA-B alleles in six highland villages (blue) and six lowland villages (red) shown on the map of Sardinia is given as a percentage (b). Of 28 alleles examined in the study only HLA-B35 shows a significantly increased frequency in the lowland (malarial) regions. These villages were chosen for study for their low levels of population migration. The allele frequency of HLA-B18 and HLA-B35 in 92 Sardinian villages or groups of villages are shown plotted against the malarial index (bottom). The index is the number of infected individuals as a percentage of the total population, measured in 1938–1940. HLA-B35 is over-represented in areas with a high incidence of malaria, while other HLA alleles are not ($r = 0.886$).

this is not so and there are many examples of polymorphisms which confer resistance or susceptibility to specific infectious diseases.

We have described several types of immune response, each of which involve the concerted interactions of many different genes. Polymorphisms occur in many of the genes involved in immune responses and the MHC class I and class II genes are the most polymorphic gene loci in humans with hundreds of different variants (Figure 5.2). Since the MHC genes are central to immune responses, it indicates that Haldane was correct in his proposal that infection has shaped the human genome, and specifically the genes that control immune responses.

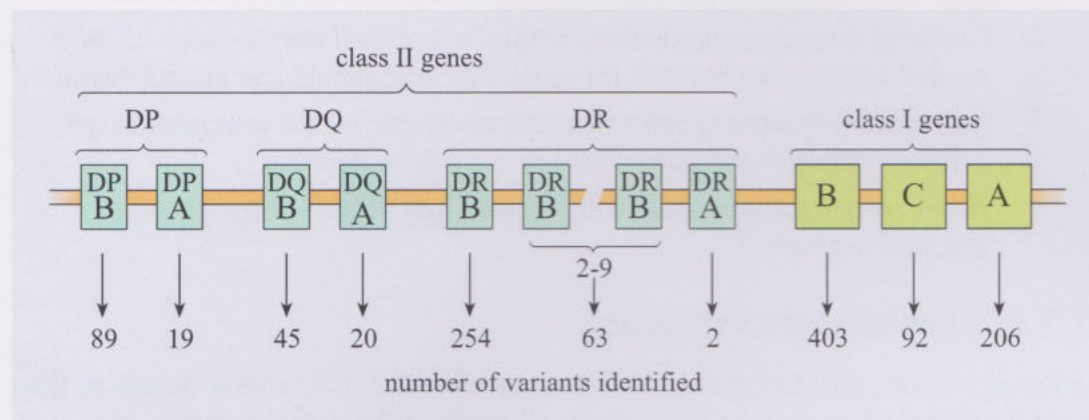


FIGURE 5.2

Polymorphism in the MHC genes. The number of genetic variants that have been identified in the different MHC gene loci (July 2000) is indicated. Notice that the MHC class II genes DP, DQ and DR are formed from two polypeptide chains α and β which are encoded by separate gene segments (DPA, DPB, etc.). The DR locus is even more variable because the numbers of genes for the DR- β chain varies between 2-9 on different chromosomes. The phenomenal amount of genetic variability is evident.

5.1.1 Mutations of genes involved in immune responses

In the simplest cases we have already identified several polymorphisms which confer disease susceptibility. These are the mutations that destroy particular proteins. They are often very informative, since they pinpoint whether a particular type of immune response is involved in defence against a particular type of infection.

- In Table 5.1 below, a number of human immune deficiencies are identified. Some of these you have encountered before and some are new. From your knowledge of the immune responses required to combat each infection, try to list the type of disease that is associated with each of the deficiencies.

TABLE 5.1 Human immune deficiencies.

Condition	Immunodeficiency	Disease type
1 MHC class-I deficiency	MHC class I and CD8 ⁺ T cells are absent	
2 Myeloperoxidase deficiency	impaired production of ROIs by phagocytes	
3 IFN γ receptor deficiency	IFN γ receptor is non functional on all cells	
4 severe combined immunodeficiency	no B cells or T cells	
5 Brutons disease	no antibodies in the serum	
6 C3 deficiency	complete absence of complement C3	
7 C9 deficiency	final step of the complement lytic pathway is absent	
8 IgA deficiency	selective lack of IgA class of antibodies	

■ The following infections are most commonly associated with these deficiencies:

- 1 Viral infections – MHC class I presents viral antigens to CD8⁺ Tc cells.
- 2 Chronic infection with bacteria – ROIs are required for killing of bacteria within the phagosome.
- 3 Severe progressive mycobacterial infection – IFN γ is required to activate macrophages which are central to antibacterial defence.
- 4 General susceptibility to all infectious pathogens, including fungi.
- 5 Susceptible to extracellular bacteria and many viruses – antibodies are required to opsonize pathogens and block their binding to host cells.
- 6 Bacterial infections, particularly staphylococci and streptococci – C3b is needed to opsonize bacteria for uptake by neutrophils and macrophages.
- 7 Neisserial infections (gonorrhoea and meningitis) – the complement lytic pathway damages the outer membrane of these bacteria.
- 8 Respiratory tract infections with bacteria and viruses – IgA protects mucosal surfaces.

5.1.2 Stable polymorphisms

Generally, when considering polymorphism, we think of more subtle variations than those described above, where the variants all produce functional proteins, but which differ slightly in their activity. The example of HLA-B35 fits into this category. Such polymorphisms affect the coding regions (exons) of the genes.

Another type of genetic variation affects how the genes are expressed, for example a variant may produce a particularly high level of a cytokine in response to stimulation. Such variants may affect the promotor (non-coding) regions of the gene in question or may affect components of the pathway which signals that the gene is to be transcribed (Figure 5.3). It is much more difficult to identify genes that produce small selective advantages or disadvantages than the wholesale deficiencies listed above.

5.1.3 Identifying the extent of the genetic component

To start to address the problem of which genes determine disease susceptibility, geneticists try to determine how great is the overall genetic component of disease susceptibility. The remaining contribution can be ascribed to environmental factors in the broadest sense. A classical approach to this problem is to measure the rate of disease *concordance* in identical twins and compare it with the incidence in the general population (concordance means that both twins have the disease). If genes alone determine disease susceptibility, then one would expect that if one twin has the disease then so will the other. If there is no genetic contribution then one would expect concordance in twins to be the same as expected from the population as a whole.

- Consider a disease which affects 10% of the population and you examine 100 pairs of twins. How many twin pairs would you expect to be concordant for the disease if the disease were wholly genetically determined? How many of the twin pairs would you expect to be concordant if there were no genetic component to susceptibility?

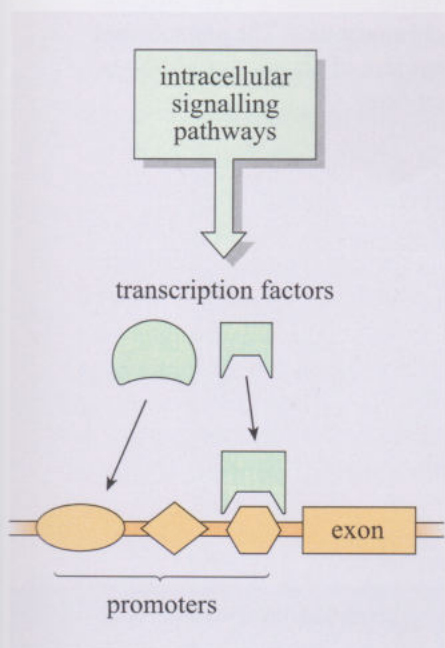


FIGURE 5.3
Polymorphism can affect the coding portion of a gene (exons) or the controlling portion of a gene which contains promotor regions that bind to transcription factors and determine how and when a gene is expressed. The intracellular signalling pathways that generate the transcription factors may also vary between individuals, and this will affect the expression of any genes that respond to those transcription factors.

- If it is wholly genetically determined, 10 pairs, that is, 1 in 10 of the twins, has the disease, and the other twin is certain to have the disease. If it is not genetic, only 1 pair of twins – it is the same as if you take any two people from the population at random. The chance of them both having the disease is the product of the incidence, that is, 0.1×0.1 or 1% of the total number of twin pairs.

Put simply, the higher the disease concordance rate in twins, the greater is the genetic contribution. Similar studies can be done with pairs of siblings, but pairs of siblings only share a proportion of their genes. Consequently concordance rates in siblings lie somewhere between the level seen in twins and the level seen in the general population.

How does this work in practice? Studies have been carried out on several diseases, including tuberculosis, malaria, hepatitis B and leprosy, all showing a major genetic component. The results are often expressed as a **relative risk**, meaning that if an individual has an affected sibling, they are more likely to contract the disease. Values of relative risk for infectious diseases typically fall between 1 and 10 (1 = no additional risk; 10 = 10 times the risk). Although these studies identify the size of the genetic contribution they do not say how many genes are involved or which ones they are. If one ignores the simple single gene immunodeficiencies, it transpires that susceptibility for most infectious diseases is determined by a number of genes and not all of the genes are equally important.

5.1.4 Identifying individual disease-risk or disease-protection genes

To identify specific disease-association genes, researchers have used genome-wide scans. They try to identify genetic markers (identifiable DNA sequences) that are associated with a particular disease. This indicates that a gene which affects disease susceptibility lies somewhere near that marker. The problem with this approach is that individual genes usually only confer slight protection or slightly increased risk. For statistical reasons, it is very difficult to identify such genes, when searching through the entire genome.

An alternative and more targeted approach is to look for polymorphisms in genes that are suspected of contributing to disease susceptibility and determining whether particular polymorphisms associate with susceptibility or resistance. Once again the idea of relative risk comes into play. A genetic variant which gives a relative risk of >1 for a disease indicates that the gene contributes to susceptibility, while a relative risk of <1 indicates a disease resistance gene. Values for individual genes in infectious diseases are often in the range 0.5–2. Although the individual genes only contribute slightly to disease susceptibility, taken together, a number of genes will add up to give a significant total genetic component. We will now look at some of the candidate genes in particular diseases.

5.1.5 Polymorphism in the MHC

The most studied gene locus in this context, is the MHC with its huge number of variant class I and II molecules. To understand the following discussion, you need to know the nomenclature for variants of MHC molecules (Box 5.1). Examples of MHC genes that are associated with particular diseases are shown in Table 5.2.

Box 5.1 Nomenclature of MHC genes

The MHC is the most polymorphic gene locus known. Originally the MHC class I and class II molecules, from the main loci (HLA-A, -B, -C -DP, DQ and DR) were distinguished using specific antibodies, which could recognize polymorphic variants at these loci. For example, HLA-A3 identifies a particular variant at the HLA-A locus. Variants defined by antibodies were called serological specificities.

Subsequently, sequence analysis of MHC-genes was undertaken and a new nomenclature developed which linked the genetic polymorphisms and the serological polymorphisms. For example, the sequences termed HLA-B*1301, B*1302, B*1303, B*1304, B*1305 and B*1306 are six different variants, all of which encode HLA-B molecules that are serologically defined by anti-HLA-B13 antibody. Following the '*', the first two digits indicate the serological specificity, and the subsequent digits indicate the sequence number associated with that specificity. Hence several genetic variants can have the same serological specificity.

To understand Section 5.1, you just need to be able to identify that a sequence such as HLA-B35 indicates a variant at the HLA-B class I gene locus.

TABLE 5.2 MHC associations with infectious diseases.

Disease	Increased risk	Decreased risk
malaria		B53, B35, DRB1* 1302
tuberculosis	DR2	DR3
leprosy	DR2	
HIV progression	B35	B27
chronic hepatitis B		DRB1*1302

Different alleles of MHC genes are associated with increased or decreased risk of developing certain diseases. The table is based on studies carried out on several distinct populations. Notice that an allele (e.g. B35) that appears to give protection against one disease can give increased risk to another.

When the variants are examined it is found that most of the polymorphism results in variations in the amino acid residues which line the antigen-binding groove (Figure 5.4).

- ☐ How does this affect antigen presentation in different individuals?
- ☒ The amino acid residues around the groove affect what antigenic peptides are bound to that MHC molecule. If individuals differ in their MHC molecules, then they will present different antigenic peptides to their T cells.

In effect, everyone's immune system is different. Some MHC molecules will be more effective at handling antigens from one particular pathogen while others will be more effective at handling another. This means that individuals will differ in their capacity to present antigens to T cells and consequently in their susceptibility to infection. Ultimately, it provides a molecular explanation for why some individuals

HLA-A2 and HLA-Aw68

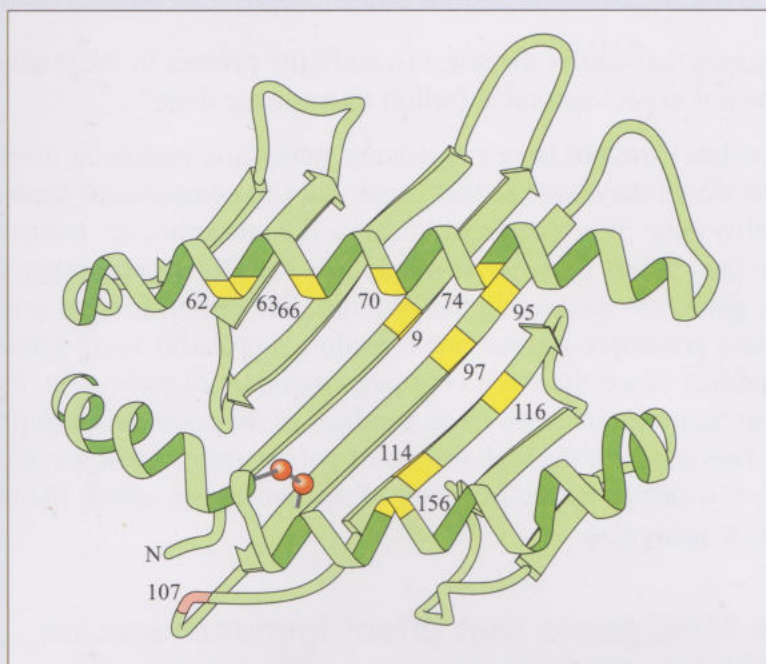


FIGURE 5.4 The diagram shows the top surface of an MHC class I molecule, looking into the antigen-binding site. MHC molecules are highly polymorphic. For example, HLA-A2 and HLA-Aw68 differ by 13 amino acids and ten of these variants are located around the antigen-binding groove, indicated in yellow. The numbers refer to the position of variant amino acids in the polypeptide chain.

are more susceptible to a disease than others. MHC genes affect the recognition of specific antigens; as you will see below, other genes can affect the ability of an individual to make different types of immune response.

It is now an appropriate time to refresh your memory of the functions of MHC molecules, and the structure of the MHC.

When you next have the opportunity, go to the animation on CD1 entitled *MHC*. As you work through this topic, consider the implications of polymorphism in the MHC and the ways in which it can affect disease. View the molecular models on MHC class I and class II structure and the expansion screens on 'The MHC peptide binding site' and 'Relative risk and MHC haplotypes'. (The term haplotype refers to the set of genes found on a single chromosome. As people are outbred each chromosome is usually different from its corresponding partner. Consequently people have two sets of different MHC genes ie two MHC haplotypes.) This table lists many autoimmune diseases that are associated with particular MHC molecule variants. All of these involve immune responses, but only a few of them are associated with infection. Many of these diseases have a high relative risk, and it has been easy to identify the associated gene. However remember that most infectious diseases have a lower genetic contribution from the MHC and in many cases the genes will be protective (relative risk <1) so it has been much more difficult to identify associations between infectious diseases and MHC variants.

After you have completed the unit, you should be able to answer Questions 1–7 of the self-assessment section.

Spend about 45 minutes on this topic.

Before going on to look at the role of other genes in disease susceptibility, it is worth addressing a question that arises from the information in Table 5.2.

- ☐ Why are genes that confer disease susceptibility present in the population at all? Would one not expect natural selection to eradicate them?
- ☒ If an individual does not have a resistance gene for a particular disease, then the variant which they have at that locus will (by comparison) appear to be a susceptibility gene. It is a statistical thing – if some genes are relatively protective then others must be relatively less so. The question then is why less protective genes are retained in the genome. The reason for this is that a gene which is less protective against one infection is probably more protective against another. Since there are very large numbers of pathogens, there is selective pressure to maintain large numbers of different genes to present their antigens. This explains the high degree of polymorphism that is maintained in the MHC. It is driven by the diversity of the pathogens, which the immune system must recognize.

5.1.6 Non-MHC genes that affect immune function

In considering non-MHC genes that alter disease susceptibility, we should first distinguish between polymorphisms that affect infectivity from those which affect the immune response. (A classical example of a variant that affects infectivity is the mutant of the chemokine receptor (CCR5 Δ 32), which you encountered in the HIV lecture.)

Consider the genes that involve immunological functions: cytokines and cytokine receptor genes have been identified for their roles in several diseases, particularly from studies involving genetically modified mice lacking the gene. There are also several suggestive studies in humans, but the results are less dramatic, because the polymorphisms are more subtle than in the gene-deleted mice.

One example in humans is a mutation in the promotor region of the gene for TNF α . A single base deletion causes the TNF α gene to be transcribed at a higher level than normal. This mutation is present in 5% of Africans and has been associated with a four-fold increase in the risk of cerebral malaria. The variant also associates with lepromatous leprosy (but not tuberculoid leprosy) and persistent hepatitis B infection. Another informative genetic association has been seen in schistosome infection in Brazil, where susceptibility was linked to a region that contains genes for IL-4, IL-5 and IL-13. You will recall that these are Th2 cytokines and the Th2 response is important in resistance against parasitic worms.

In addition to the examples above, several other polymorphisms have been associated with disease susceptibility. Some of these could involve the immune response, but others probably affect more general aspects of infection and others may be incorrect assignments due to the methods used. A problem which has arisen many times is that the findings of one research group are not always readily replicated by others. Although this may be due to the kind of incorrect assignments that occur when large numbers of genes are tested, another explanation is possible. We have noted that disease susceptibility is dependent not just on the individual genes, but also the strain of a pathogen which varies with location, and other interacting genes which vary with ethnic group. Therefore what may be a disease susceptibility gene for a pathogen in one context, may have no detrimental effect or

even promote resistance in a different ethnic or geographic context. Such complex genetic interactions are often seen in diseases where several genes contribute to resistance or susceptibility.

5.2 Strategies of immune evasion

We started this book with a broad view of the relationship between host and pathogen, asserting that pathogens had driven the evolution of the immune system and vice versa. We have seen evidence that pathogens have been and continue to be instrumental in the evolution of different types of immune defence.

Having seen the range of host defences, we now come to the mechanisms that the pathogens use to evade immune responses. This is the other side of the coin. Immunologists have now identified literally hundreds of examples of mechanisms that pathogens have evolved to avoid destruction by the immune system. Two general principles can be extracted from these data:

- All pathogens deploy mechanisms to evade immune responses, and their evasion strategies are selectively directed at just those elements of the immune system which act against the pathogen.
- Any element of the immune system may become a target for an evasion strategy. Different pathogens subvert antibody responses, complement defences, cytotoxic T cells, NK cells, antigen presentation, immune recognition, cytokine mediated intercellular signalling, intracellular signalling pathways and the generation of toxic molecules.

From this we can infer that immune evasion is absolutely necessary for the survival of pathogens, and the only pathogens which we see today are here because they have evolved ways of evading immune responses.

We can distinguish two main categories of immune evasion:

- 1 antigenic variation and disguise;
- 2 countermeasures against the immune effector mechanisms.

These two categories can broadly be related to the two phases of an immune response, firstly recognition of the pathogen by lymphocytes, followed by activation of appropriate systems to destroy it.

Many pathogens evade both recognition and destruction, but the more sophisticated systems tend to be confined to larger organisms with big genomes. For example HIV (a small RNA virus) uses antigenic variation and synthesizes two proteins that interfere with antigen presentation and immune recognition. In contrast, *Herpes simplex* virus (a large DNA virus) produces many proteins that interfere with practically every aspect of the immune response.

5.2.1 Antigenic variation

Two of the viruses examined in the case studies (HIV and influenza) are good examples of pathogens that use antigenic variation. Notice, however, that the way they do this is quite different. Influenza produces an acute infection with a single strain of virus in one individual. The genetic variation is seen on a global scale, with different variants present in different parts of the world. By contrast, HIV

establishes a chronic infection with mutants developing within the individual, and the co-existence of different variants in one person. Such diseases present problems for immunologists who aim to develop vaccines.

- ☐ What strategies might be adopted in preparing a vaccine against a virus that undergoes antigenic variation?
- ☒ Strategies might try to (a) identify parts of the virus which do not vary, and (b) produce a vaccine that induces an immune response (antibody or T cell) recognising that part of the virus. This is one of the approaches that have been tried for HIV. The strategy used for influenza is quite different and involves a global screening programme to identify new variants as they arise and prepare vaccines against them before an epidemic develops.

5.2.2 Trypanosomiasis mini-lecture (CD2)

As another example of extreme antigenic variation, we shall look at the disease sleeping sickness, which is caused by a parasite, *Trypanosoma brucei*, which affects cattle and people in Africa. This is a protoctist that lives in the blood and evades the immune response by changing its antigens.



You should now go to the mini-lecture entitled *Trypanosomiasis* on CD2. The immune defences against this parasite are relatively simple, as they are principally mediated by antibodies and macrophages. However, the way in which the parasite evades the antibody response is sophisticated and highly effective.

You should spend about one hour on this activity.

After you have worked through the lecture, you should be able to answer the following questions (the answers are given at the end of the book).

Question 5.1

How are trypanosomes cleared from the blood and how quickly after infection?

Question 5.2

Why does an infected person develop successive waves of parasitaemia?

Question 5.3

What are variant surface glycoproteins (VSGs)?

Question 5.4

How much of the parasite's genome is devoted to encoding VSGs?

Question 5.5

How many different VSGs does a parasite express at one time?

Question 5.6

What molecular mechanism allows the parasite to change its surface antigens?

When we compare trypanosomiasis with viruses that undergo antigenic variation, there is a fundamental difference in the mechanisms used. The trypanosomes have a relatively large genome, sufficient to have up to 1000 genes for variants, so any parasite carries with it a large set of variant genes, and can switch between them. However, the genome itself is relatively stable. By comparison, viruses mutate or recombine their genes. Any virus generally only carries one copy of the genes for its surface proteins and antigenic variation involves replacement of one strain with another. However, as viruses are comparatively genetically unstable, this mechanism for mutation and antigenic variation is well developed.

5.2.3 Antigenic disguise

A different strategy for evading immune recognition is adopted by schistosomes and we call it **antigenic disguise**. When schistosomes first enter a new host they are highly susceptible to damage by antibodies and complement, but this is lost after a few hours. The process is illustrated in mice that have been infected with schistosomules (Figure 5.5). During the first few hours, schistosomules bind antibodies which recognize parasite antigens, but the ability of the antibodies to recognize the parasite rapidly diminishes. Concomitantly, the parasites take up molecules from their environment, which cloak their own surface antigens. Host blood group antigens can be detected on the parasite surface together with MHC molecules and other host proteins. It is thought that this masking prevents damaging parasite-specific antibodies from reaching the cell surface to activate complement.

5.2.4 Malaria (CD2)

Another aspect of antigenic variation is seen in parasites that have complex life cycles. From the case study on malaria in Book 1, you will appreciate that the malarial parasite progresses through several different stages – sporozoite, schizont, merozoite, etc. The antigens expressed at each stage of the life cycle differ from each other, which means that different immune recognition patterns are required at each stage of infection and different immune effector mechanisms are appropriate for each stage.

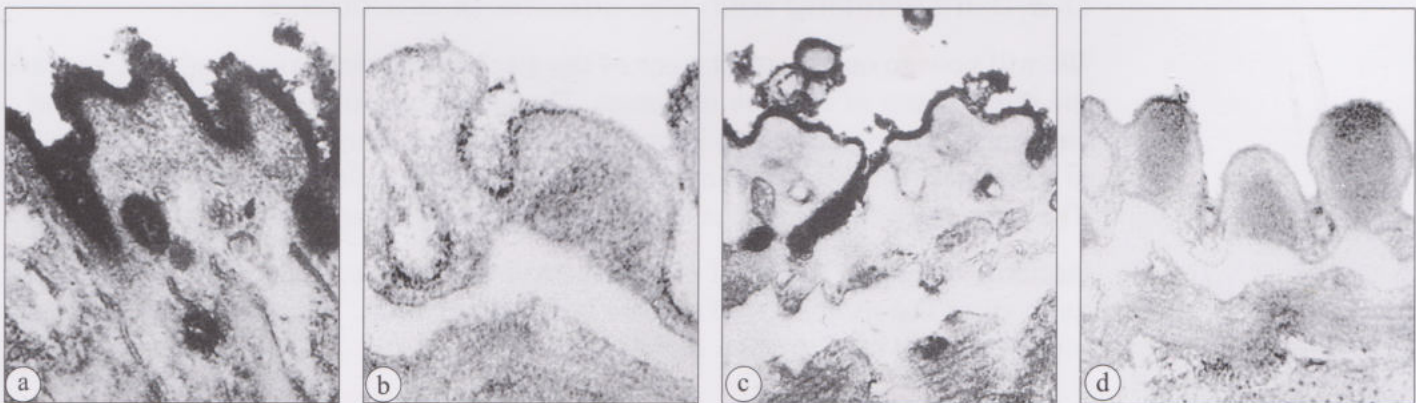


FIGURE 5.5 The antigenic disguise of schistosomes. The electron micrographs show sections of the surface of schistosomes labelled to identify surface antigens, which appear as electron-dense deposits. (a) A 3-hour-old schistosomule labelled with antibody to schistosomule antigens. (b) A schistosome that has been 4 days inside a mouse host labelled with anti-schistosomule antigens has lost its specific antigens. (c) A 4-day-old parasite labelled with antibodies to mouse erythrocyte antigens shows that the parasite has acquired mouse antigens on its surface, whereas the newly infected schistosomule (d) lacks the mouse antigens. Newly infected schistosomules are susceptible to antibodies and complement, while older ones are not.



You should now go to the mini-lecture on CD2 entitled *Malaria*. This will remind you of the stages of the malarial life cycle and correlate them with the different types of immune defence available. When we come to consider vaccination against malaria in Book 7, it is important to consider not just what stage of the life cycle is an appropriate target, but also which antigens could be targets for an immune-mediated attack.

Spend about one hour on this activity.

After you have listened to this mini-lecture, you should be able to answer the following questions (the answers are given at the end of the book).

Question 5.7

What types of immune defence are appropriate for dealing with sporozoites?

Question 5.8

What immune defences can recognize parasitized red cells?

Question 5.9

How does *Plasmodium falciparum* produce the symptoms of cerebral malaria?

Question 5.10

What role does the circumsporozoite antigen have in evading immune responses?

Question 5.11

Do T cells have any role in protective immunity against malaria?

Question 5.12

What evidence is there for genetic polymorphisms affecting susceptibility to malaria?

5.2.5 Interfering with the actions of antibodies

We will now go on to look at some of the mechanisms that have developed to evade the effector arm of immune responses. There are numerous examples of different pathogens that interfere with immune responses, but viruses are particularly adept at this, since their life cycle involves them subverting cellular signalling processes. The examples given cover the entire range of immune reactions.

Bacteria, including strains of staphylococcus and streptococcus, have receptors for antibodies. The receptor on *S. aureus* is called protein A. These receptors bind with high affinity to the Fc region of IgG antibodies.

- ☐ At first sight it would seem totally counterproductive for a pathogen to bind an antibody. On reflection, can you see how such a receptor could help the bacterium survive?

- By binding to the Fc portion of IgG, it coats the bacterium with a host molecule. Moreover, if the Fc portion is bound to the bacterium, then it cannot bind to Fc receptors on macrophages. Nor can it bind and activate the complement classical pathway. This means that two critical pathways involved in host defence are less effective (Figure 5.6).

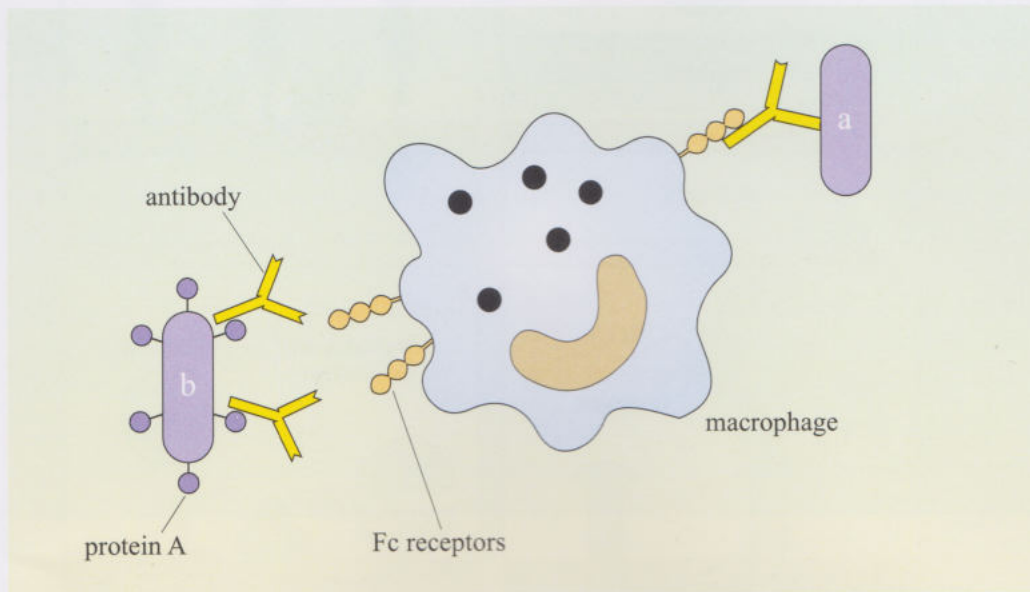


FIGURE 5.6

Normally macrophages can recognize antibodies bound to the surface of bacteria using their Fc receptors (a), but staphylococcus strains expressing protein A bind to the antibody's Fc region, thereby preventing it from binding to the Fc receptors on the phagocyte (b).

Fc receptors are also found on some viruses, particularly those such as *herpes simplex* (HSV-1), which establish chronic infections. These viruses must survive in a host that has high levels of specific anti-viral IgG. So these types of defence are at a higher premium in chronic virus infections than in acute infections, where the virus can complete its replication cycle before the immune response has switched to IgG production.

Another way of evading antibody responses is by the release of decoy proteins. These are molecules released from the pathogen surface or from the surface of an infected cell that can bind and mop up free antibody. As immune complexes form away from the surface of the pathogen, macrophages, complement and NK cells are less likely to be able to bind to the surface antigen.

- Can you recall any examples of pathogens that release such proteins?
- The circumsporozoite antigen of malaria acts as a decoy protein.

5.2.6 Evasion of complement-mediated defences

Bacteria employ a number of strategies to avoid complement-mediated damage (Figure 5.7). Bacterial capsules are generally poor activators of the complement alternative pathway, and they do not bind antibodies well. This means that complement C3b does not become deposited on the bacterial surface, resulting in poor phagocytosis by macrophages.

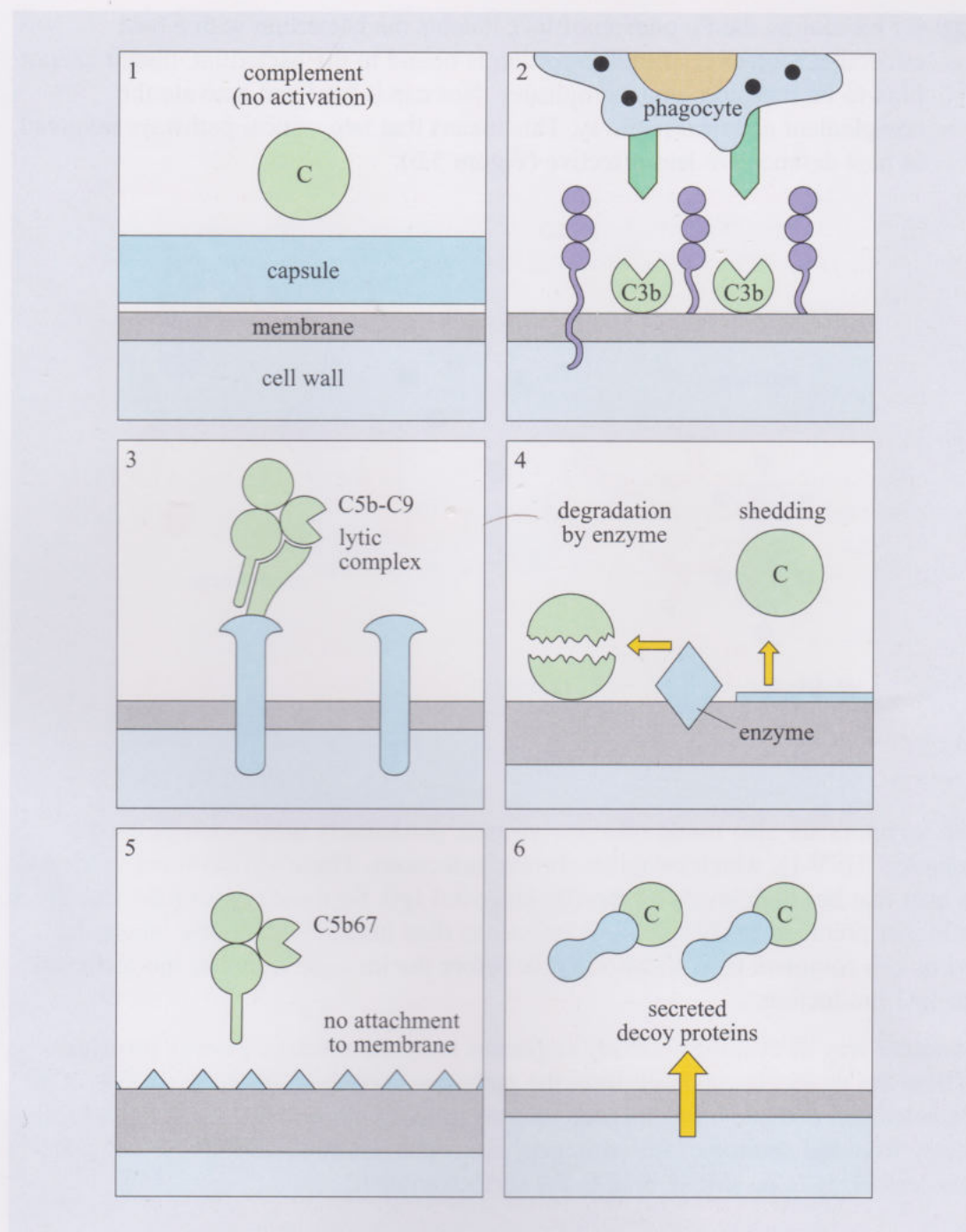


FIGURE 5.7 Bacteria use a variety of strategies to avoid complement mediated damage. (1) Many bacteria have an outer capsule that inhibits complement activation. (2) Some have structures which prevent phagocyte C3 receptors from accessing bound C3b on the bacterial surface. (3) Some have proteins which bind to complement components at a distance from the cell surface where they can do little damage. (4) Surface enzymes can lead to the enzymatic degradation of bound complement molecules. (5) To initiate lysis a complement intermediate C5b67 must first attach to the membrane. If this is prevented, the lytic membrane attack complex cannot assemble. (6) Several species secrete proteins that mop up complement components or can bind and neutralize the complement chemotactic fragment C5a.

An alternative strategy is for bacteria to cause complement activation, but at a distance from the cell surface, where membrane attack complexes are ineffective. They can also use a similar strategy to the one noted above; they produce decoy proteins, which cause complement activation away from the pathogen.

Viruses too can evade complement-mediated defences, but they generally borrow the host's own protection mechanisms. You will recall that cells of the body produce a number of proteins which are expressed on the cell surface and which limit the local activation of complement by the alternative pathway and promote the breakdown of the enzyme which activates C3 (Section 2.3.1). These molecules are typically inserted into the plasma membrane of the cell. Other members of this family of molecules promote the breakdown of C3b. Such proteins have been borrowed and modified by pox viruses and herpes viruses, to limit complement activation.

- ☐ At what stages in the life cycle of the virus would such proteins be protective for the virus?
- ☒ They may be inserted into the membrane of an infected cell to prevent it from being lysed by complement or targeted by cells that have C3b receptors (macrophages and NK cells). Additionally, they can prevent complement-mediated attack and opsonization of enveloped viruses as they move between cells.

Another molecule that is present on the surface of mammalian cells is CD59, also called protectin. It acts to shed membrane attack complexes from the plasma membrane. A herpes virus containing homologues of this protein has been identified. Notably, the amino acid sequence of this viral protein shows 50% sequence homology with human CD59 and 70% homology with that from a monkey. This clearly shows that viruses can collect genes from the host they infect and use them for their own defence.

5.2.7 Resistance to intracellular killing mechanisms

In the lecture on tuberculosis the high resistance of the bacteria to intracellular destruction was emphasized. Mycobacteria secrete molecules which inhibit the assembly of the proton pump required for the production of reactive oxygen intermediates, while the outer coat of *M. leprae* contains phenolic glycolipids which scavenge the free oxygen radicals. Other proteins from *M. tuberculosis* inhibit fusion of lysosomes with the phagosome. Interestingly, some viruses also deploy proteins that limit the damage caused by reactive oxygen intermediates, and this suggests that some viruses, as well as bacteria, may be susceptible to damage by these agents within the phagosome. It is hard to think of a different explanation for them retaining enzymes that neutralize reactive oxygen intermediates.

5.2.8 Interference with antigen presentation and MHC molecule expression

Antigen presentation involves many steps, all of which are needed for expression of viral antigens on the cell surface. It is therefore unsurprising that viruses have evolved mechanisms to interfere with these processes. Some of the points of action are shown in Figure 5.8. For example, both cytomegalovirus and *herpes simplex* interfere with the transport of antigenic peptides from the cytoplasm to the endoplasmic reticulum. Cytomegalovirus also produces proteins that keep MHC class I molecules in the endoplasmic reticulum and ultimately target them for degradation. The protein Vpu from the human immunodeficiency virus destabilizes newly synthesized MHC class I molecules while Nef causes MHC molecules on the

cell surface to be rapidly endocytosed. Although different viruses are interfering with antigen presentation at different points, any stage of the process appears to be susceptible to some virus.

Before studying this section you may find it helpful to review the normal process of antigen presentation in Figure 2.30.

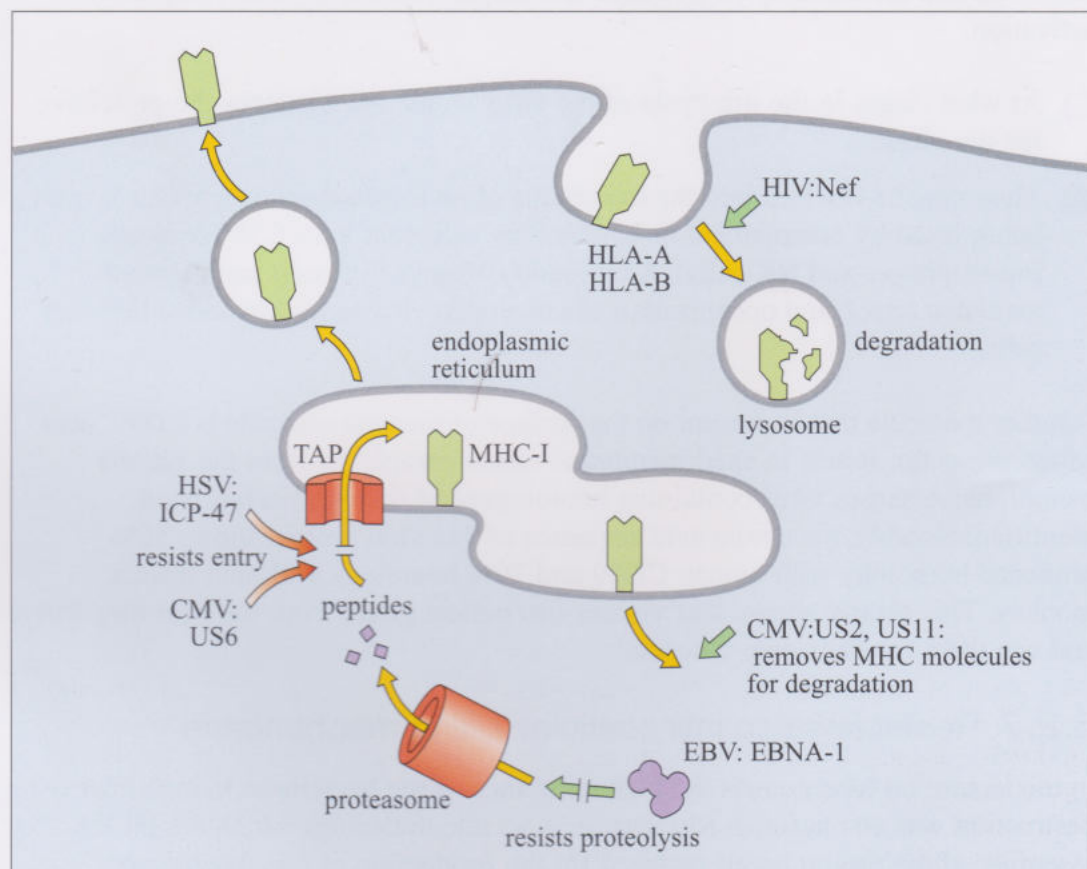


FIGURE 5.8 Different viruses can inhibit different parts of the antigen-presentation pathway. Examples are shown. Epstein Barr virus (EBV) produces some proteins (EBNA-1) which are particularly resistant to processing by the proteasome. Herpes simplex virus (HSV) and cytomegalovirus (CMV) produce proteins that interfere with the transport of antigenic peptides into the endoplasmic reticulum. CMV also produces proteins which remove MHC class I from the endoplasmic reticulum and promote its degradation. HIV produces a protein Nef, which accelerates the recycling of MHC molecules from the cell surface and directs them for degradation by lysosomes.

One problem with this approach for the virus is that loss of MHC class I molecules should make the infected cell particularly susceptible to lysis by NK cells (see Figure 2.48). However, herpes viruses in particular have further countermeasures to evade NK cells. Cytomegalovirus produces a bogus MHC class I molecule, to replace the one which has been targeted for degradation. The bogus molecule does not bind or present antigenic peptides, therefore it does not mediate immune recognition by Tc cells. However it is good enough to fool NK cells into believing that the cell is expressing MHC class I molecules. It binds to their killer inhibitory receptors, thereby causing them to leave the cell alone. By using these two complementary defence mechanisms, cytomegalovirus can evade the body's two principal mechanisms for destroying virally-infected cells, that is, Tc and NK cells.

Evasion of recognition is, however, only one of several mechanisms that have allowed herpes viruses to become so successful as latent pathogens (Figure 5.9).

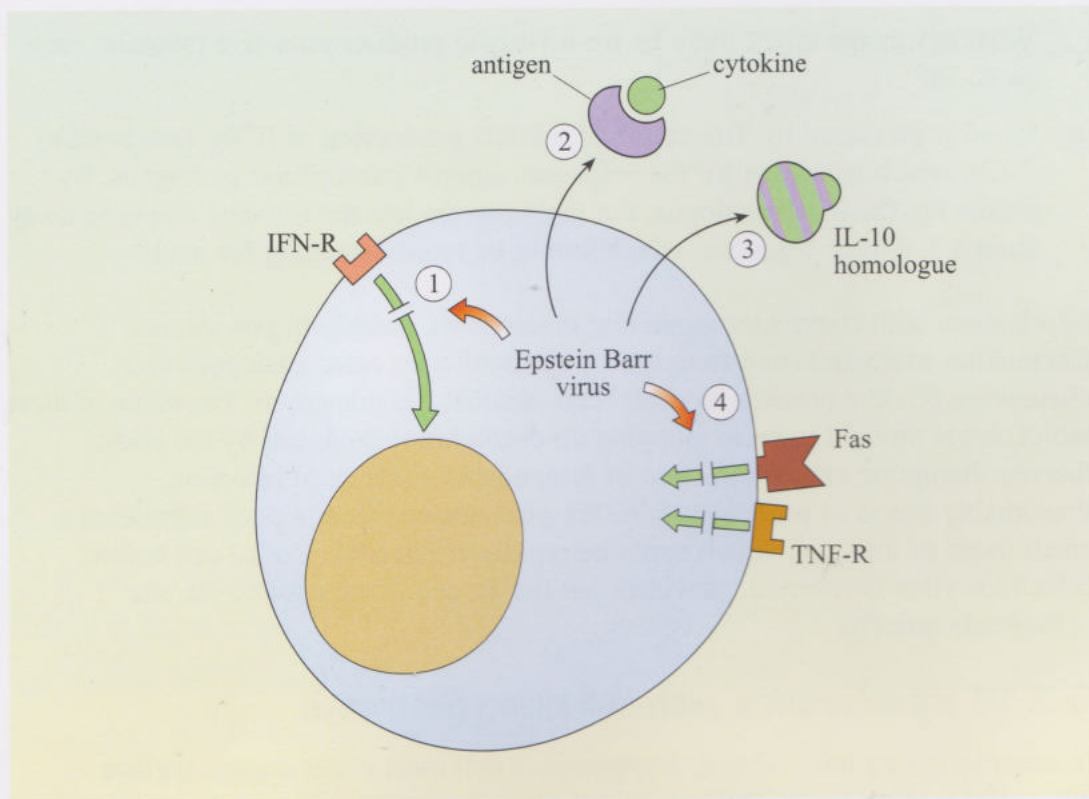


FIGURE 5.9 Mechanisms by which Epstein Barr virus (a herpes virus) can interfere with immune responses. Different proteins carry out each of the functions. (1) Interference with IFN-mediated signalling, so that production of anti-viral proteins and MHC molecules are not upregulated. (2) Production of secreted molecules which bind to a cytokine (M-CSF) required for macrophage development. (3) Production of a homologue of IL-10 that deviates the immune response away from the TH1-type. (4) Production of factors that interfere with the cell death pathways (see Figure 2.50) and thereby promote survival – these molecules are homologues of the ones that B cells normally use to promote survival of selected cells as they develop in the germinal centres.

5.2.9 Interference with cytokine-mediated signalling

Cytokine signals are essential for development of effector responses against pathogens. The signal from a cytokine can be intercepted as the cytokine passes through the extracellular milieu, or the signals from the cytokine receptor can be blocked at the receptor or in the intracellular signalling pathways.

- ☐ What cytokines would you expect intracellular pathogens to target?
- ☒ Interferon- γ and IL-12 are most important in activating the TH1 response and these are targeted by several intracellular pathogens.

For example, *M. tuberculosis* interferes with intracellular signalling from the IFN γ receptor, to delay and reduce macrophage activation, which is required to kill the phagocytosed bacteria. Aiming at the same arm of the immune system the parasite *Leishmania* sp. and measles virus both inhibit the production of IL-12 by macrophages.

A different approach is taken by cytomegalovirus. It synthesizes an analogue of IL-10, which can be secreted by an infected cell. Unlike the analogues of MHC molecules mentioned above, this appears to be functional.

- ☐ What advantage might there be for a virus to produce an active cytokine such as IL-10?
- ☒ IL-10 is produced by T_H2 cells and inhibits production of $IFN\gamma$ (see Section 2.12), which is needed for the responses against intracellular pathogens. By producing the IL-10 analogue, the virus can deviate the immune response away from a T_H1 -type response, which would be more damaging for itself.

Interference with chemokine signalling is also seen, notably in pox viruses. Chemokine analogues (non-functional), chemokine receptor analogues and chemokine-binding proteins have all been identified in this group. The value of these molecules is thought to be in mopping up chemokines produced by the body, thereby disrupting chemoattraction of lymphocytes to sites of infection. Presumably this is of particular value for pox viruses where a pock represents a small focus of infection which could be rapidly eradicated by a T_c cell before infectious virus is released, provided that the T_c cell is attracted to the site sufficiently quickly.

5.2.10 Interference with cell killing pathways

In order to kill an infected cell, a cytotoxic T cell must either engage surface molecules such as Fas (CD95) signal via cytokines or release granule-associated mediators. Any virus that can interfere with signalling at the cell surface, or prevent the cascade that leads to the production of caspases and hence to apoptosis, is clearly at an advantage. One approach to this is to place non-functional TNF receptor analogues on the cell surface, while another is to internalize and degrade Fas. In each case the loss of the functional receptor on the infected cell means that it cannot receive a signal to activate the signalling pathways leading to apoptosis. An alternative approach adopted by vaccinia virus is to produce an enzyme inhibitor which blocks the action of caspase-8 itself, central to these pathways.

A third mechanism is to provide the cell with increased cell survival signals, which can counteract progression to apoptosis. An example of this is seen in glandular fever, caused by Epstein Barr virus (EBV). Recall that B cells in the germinal centres will die by apoptosis *unless* they are rescued by signals from T helper cells and B cells receive the survival signal through a surface molecule called bcl-2. Epstein Barr Virus, which infects B cells, has two homologues of bcl-2. When a B cell is infected by EBV these molecules will transduce signals for B cell survival and one of the main features of EBV infection is B cell proliferation.

5.3 Evolving hosts: evolving infections

The variety of ways in which pathogens aim to evade immune responses demonstrates that evasion is essential for the survival of the pathogen. Indeed, the difference between what we consider to be a pathogen and a harmless microorganism can be related to the countermeasures employed by pathogens. 'Harmless organisms' can also produce disease, but only in individuals with

impaired immune systems, where their inability to evade the appropriate immune response is not a disadvantage.

In describing the different types of immune response and the countermeasures which pathogens have developed, we can see that an evolutionary race has developed between the infectious diseases and the immune system of their hosts.

There is good evidence that the development of new pathogens has led to the evolution of new immune defences to counter them. Conversely, the development of evasion strategies by pathogens has principally been driven by immune responses in the host population and immunity in general. The picture that we see currently of pathogens and their hosts is a just a snapshot. There is no reason to suppose that the process has stopped and every reason to infer that new pathogens will continue to arise causing further evolution of the immune system.

Summary of Chapter 5

- 1 Large numbers of genes are involved in controlling immune responses and in determining whether an individual is more or less resistant to a particular infection. Of these, the MHC genes are particularly important because they affect which antigens an individual presents to their T cells, and because they are highly polymorphic within the population.
- 2 Individual MHC genes can result in increased or decreased risk of infection, depending on the infectious agent involved. The MHC genes present in a population can be related to the infectious agents that are prevalent in that population.
- 3 Genes affecting antigen presentation, cytokine production, and numerous other immunological functions also can affect disease susceptibility.
- 4 Pathogens have evolved numerous ways of evading immune responses. This is usually very selective, so that pathogens deploy countermeasures against just those immune responses that act against themselves.

Learning outcomes for Chapter 5

- 5.1 Define and use, or recognise definitions and applications of, each of the terms printed in **bold** in the text.
- 5.2 Explain some of the ways in which genetic diversity in humans can lead to increased susceptibility or resistance to infection.
- 5.3 Explain why genes that protect against one organism can increase susceptibility to another. Explain how stable polymorphism in the MHC genes can be related to the infectious diseases prevalent in a particular area/ population.
- 5.4 Give examples of the ways in which pathogens have evolved in order to evade immune recognition or immune effector mechanisms.
- 5.5 Understand how the immune system exerts selective pressure on populations of pathogens, causing them to mutate and develop within an individual and within populations, using HIV and influenza as primary examples.

SUMMARY OF BOOK 3

- 1 The immune system has evolved different ways of protecting the host against different pathogens. The innate defences act in concert with adaptive immune responses mediated by lymphocytes. The characteristics of an adaptive immune response are specificity and memory.
- 2 Recognition of pathogens and their antigens is carried out by lymphocytes, which fall into two main subsets: B cells use surface antibody to recognize intact antigens in solution and on the surface of infected cells. Eventually they differentiate into plasma cells that produce a secreted form of the antibody. T cells use their T cell receptor to recognize processed antigen fragments presented by MHC molecules on the surface of other cells of the body.
- 3 Cytotoxic T cells recognize antigen presented by MHC class I molecules on the surface of infected cells. Any nucleated cell can present antigen to a cytotoxic T cell. A cytotoxic T cell can then signal the infected cell to die by apoptosis.
- 4 Helper T cells fall into two categories; Th1 cells release cytokines which promote the activation of macrophages. Th2 cells release cytokines which promote antibody production (and the action of eosinophils). Helper T cells interact with a restricted group of antigen-presenting cells, which include B cells, macrophages and dendritic cells.
- 5 Whether an individual makes a Th1-type response or a Th2 type of response to a pathogen depends on their genetic make-up and on how the antigens are first presented to the lymphocytes. Th1 cells tend to turn off Th2 cells and vice versa.
- 6 NK cells recognize and react against infected cells that have down-regulated their MHC class I molecules. When NK cells use antibody to recognize infected cells, this is called K cell activity.
- 7 Antibodies specifically bind to antigens on a pathogen and may interfere with its ability to attach to and infect host cells.
- 8 Different classes of antibody have different functions. IgA is secreted across mucosal membranes, and is important in protecting these sites against pathogen entry. IgG and IgM antibodies promote phagocytosis, either directly, or by activating the complement system. IgE is important in the development of inflammation.
- 9 Individual B cells start by producing IgM, but can later switch to produce other antibody classes. This change is reflected in the antibodies present in the blood as an immune response develops. Antibody affinity rises during an immune response as IgA, IgG and IgE are produced.
- 10 The complement system is a group of molecules, present in blood and tissue fluids, which can be activated either by immune complexes or by the surface of many pathogens, and which lead to pathogen destruction and/or the activation of inflammation.
- 11 Inflammation is a process that brings cells and molecules of the immune system to tissues at sites of infection or cell damage.

- 12 Phagocytes are important in defence against many bacterial infections. They phagocytose pathogens and subject them to a barrage of toxic reactive oxygen and nitrogen compounds. Killed bacteria are digested by lysosomal enzymes.
- 13 Leukocytes interact with each other and with cells of the body by direct cell/cell interaction and by the release of cytokines. Cytokines fall into different families and each cytokine has a specific function, acting only on cells with the appropriate receptor. Cytokines such as IL-1 and TNF are responsible for some disease symptoms, such as fever and appetite loss.
- 14 Immunological memory is a key component of long-lasting immunity to an infectious agent. The basis of memory is the proliferation of lymphocytes during the primary immune response. Some of these cells become very long-lived memory cells, which have differentiated, so that they are more readily activated following a second encounter with the same antigen.
- 15 Cytotoxic T cells and NK cells are important in clearing virally infected cells. Antibodies can limit viral spread in the extracellular spaces, and interferons are important in limiting the ability of virus to replicate within cells.
- 16 In immunosuppressed individuals, organisms which are normally harmless may become pathogenic.
- 17 Some infections lead to secondary diseases such as tumours or hypersensitivity reactions, which are more damaging than the initiating infection. Cross-reactive antigens on pathogens can lead to autoimmune diseases.
- 18 Large numbers of genes are involved in controlling immune responses and in determining whether an individual is more or less resistant to a particular infection. Of these, the MHC genes are particularly important because they affect which antigens an individual presents to their T cells, and because they are highly polymorphic within the population.
- 19 Pathogens have evolved numerous ways of evading immune responses. This is usually very selective, so that pathogens deploy countermeasures against just those immune responses that act against themselves.
- 20 The immune system appears complex, because it has to respond against a wide variety of pathogens and defence systems have evolved which are appropriate for each pathogen.

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ANSWERS TO QUESTIONS

QUESTION 2.1

There are several different types of flu virus and the virus mutates and recombines to generate new strains at regular intervals. The immune response is specific for a particular strain – last year's immune response does not protect against this year's strain of flu.

QUESTION 2.2

Antibodies recognize epitopes – molecular shapes formed by combinations of amino acid residues (sometimes also lipids and carbohydrates) – and form multiple non-covalent bonds with them. The antigen and antibody may be thought to have complementary molecular shapes. A mutation in a flu virus that produces a change in an epitope means that the antibody and antigen no longer have complementary shapes. The antibody therefore binds less well to the new strain of virus, and is less effective in preventing the spread of infection.

QUESTION 2.3

The haemagglutinin is on the outside of the virus and accessible to antibodies. The M-protein is inside the intact virus, and therefore not accessible to antibody.

QUESTION 2.4

The epitopes of the haemagglutinin mutate which allows them to avoid recognition by preformed antibodies. Mutation of some parts of the haemagglutinin is restricted, since the molecule must perform its function of attaching to glycoporphins. The M-protein does not mutate because the protein must perform its structural functions within the virus, and there is no selective pressure for it to mutate, since antibodies against the M-protein do not protect against disease.

QUESTION 2.5

A major pandemic strain is produced by recombination of genome segments from different strains of flu. Often, several of the viral proteins/antigens will be different in a new pandemic strain. For example, both the haemagglutinin and the neuraminidase may change. This means that there is little residual immunity provided by antibodies against an antigen from a previous strain. Minor changes in an antigen (antigenic drift) may result in reduced antibody binding (lower affinity), while not completely destroying binding. Major changes in an antigen (antigenic shift) completely prevent antibody from recognising the new strain.

Also, new pandemic strains may have changed viral enzymes, which allow faster viral replication in the host.

QUESTION 2.6

Vaccine scientists try to anticipate what strains of flu will present the major health threat in any one year. Firms then manufacture vaccine using these strains. If the scientists have got it right, the vaccine will be protective, but if they get it wrong, or a new strain arises in the interim, the vaccine will be less effective or ineffective.

QUESTION 2.7

A deficiency of IFN γ or IL-12 predispose the individual to mycobacterial disease. These cytokines are central to the TH1-type of immune response.

The gene NRAMP1, which determines resistance to mycobacteria, is expressed in macrophages.

QUESTION 2.8

Reactive oxygen intermediates, reactive nitrogen intermediates and lysosomal enzymes are active against mycobacteria. Limiting the availability of divalent cations is also important.

QUESTION 2.9

IFN γ , IL-12 and TNF α .

QUESTION 2.10

The HEAF test is commonly used. Antigen from mycobacteria is pricked into the skin: if the individual has been exposed to tuberculosis their T cells react against it and attract macrophages to the site producing a raised area of inflammation in the skin.

QUESTION 2.11

Susceptibility to TB depends partly on the person's genetic profile and their general state of health, including nutritional status and the presence of other infections. These all affect the functional efficiency of the immune system in controlling mycobacterial infection.

QUESTION 3.1

It attaches using the cell surface molecule CD4 and a chemokine-receptor (usually CCR5 or CXCR4).

QUESTION 3.2

T-tropic viruses preferentially use CXCR4 as their co-receptor, while M-tropic viruses use CCR5.

QUESTION 3.3

Some individuals have a mutation of their CCR5 gene. This means that M-tropic strains of the virus, which are infectious and predominate in the earliest stages of the infection, cannot spread quickly.

QUESTION 3.4

Antibodies and cytotoxic T cells.

QUESTION 3.5

The viral reverse transcriptase has a very high error rate, consequently the virus mutates rapidly. Also the virus targets cells of the immune system, which are involved in host defence.

QUESTION 3.6

Opportunistic infections, that normally do not trouble healthy people. Examples are candidiasis, pneumonia caused by *Pneumocystis carinii*, toxoplasmosis and herpes infections. Tuberculosis is also much more common in AIDS patients than in the general population.

QUESTION 3.7

This group of infections is normally controlled by cell-mediated immunity mediated by CD4⁺ T cells, particularly TH1 cells. The loss of this population of lymphocytes allows the opportunistic infections to spread.

QUESTION 4.1

hydatid cysts	type I hypersensitivity
hepatitis B	type III hypersensitivity
leprosy and schistosomiasis	type IV hypersensitivity
streptococci	rheumatic fever
campylobacter	Guillain Barré syndrome

QUESTION 4.2

Type I hypersensitivity reactions occur in individuals who have a high level of IgE to the antigen. Mast cells bind IgE on their Fc receptors and are then triggered to release their inflammatory mediators, following contact with the antigen.

Type II hypersensitivity reactions occur when IgG or IgM antibodies bind to the surface of cells or extra-cellular matrix components, causing the accumulation of phagocytes and the local release of toxic molecules.

Type III hypersensitivity reactions occur when the body has large loads of antigen to deal with. Immune complexes are formed in large amounts – so great that it exceeds the capacity of the mononuclear phagocytes to remove them from the blood stream. The complexes often become deposited at sites of filtration, especially in the kidney and this leads to inflammatory damage in that organ.

Type IV hypersensitivity reactions are mediated by T cells and mononuclear phagocytes. T cells are sensitized to an antigen, and on encountering that antigen release cytokines that cause the accumulation of macrophages. The damage is mediated by cytokine-activated macrophages.

QUESTION 4.3

A cross-reaction between a pathogen molecule and a host molecule. The immune system recognizes and mounts an immune response against the pathogen, which coincidentally is directed against the host. Costimulation of T cells breaks tolerance to cross-reactive molecules because the antigens of the pathogen are presented to the immune system in a different way to self molecules.

QUESTION 5.1

They are cleared by antibody and phagocytes within 1–2 weeks of infection.

QUESTION 5.2

The parasite changes its surface glycoprotein, and antibody against the earlier variants is ineffective against the new variant.

QUESTION 5.3

The VSGs are glycolipid-anchored antigens which cover the surface of the trypanosome and protect it from phagocytosis.

QUESTION 5.4

There may be up to 1000 genes for different VSGs – a significant proportion of the genome.

QUESTION 5.5

Only one.

QUESTION 5.6

The new VSG is moved into an active transcription site by a process of gene conversion, i.e. swapping the old gene for the new gene.

QUESTION 5.7

Antibodies can intercept sporozoites in the bloodstream.

QUESTION 5.8

Antibody-dependent cell-mediated cytotoxicity effected by large granular lymphocytes (K cells). The parasitized red cells may also be targeted by antibody against parasite antigens on their surface, and complement.

QUESTION 5.9

Antigens of *P. falciparum* insert into the membrane of the parasitized red cell and this causes it to attach to endothelial cells in the blood vessels of the brain. This can cause local obstruction of the vessels, but also induces local inflammation.

QUESTION 5.10

CS antigens are shed in the presence of serum containing specific antibodies, thereby acting as a decoy.

QUESTION 5.11

Yes, both CD4⁺ and CD8⁺ T cells are involved, possibly by releasing IFN γ and activating macrophages. If T cells had no role, then one would not see the genetic association with particular MHC haplotypes.

QUESTION 5.12

Human populations in endemic malaria areas have a higher proportion of MHC haplotypes that confer immunity, than in non-endemic areas (e.g. HLA-B53). The sickle cell anaemia gene is also partially protective in people who are heterozygotic and this helps to maintain an otherwise disadvantageous gene in the population of malarial areas.

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Figures

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INDEX

Note: Entries in **bold** are key terms. Page numbers referring to information that is given only in a figure or caption are printed in *italics*.

A

- acquired immunodeficiency syndrome (AIDS) 107–8
- ADCC *see* antibody-dependent cell-mediated cytotoxicity
- adenoviruses 96
- adhesion molecules 43–4, 55, 75, 82
- adjuvants** 78
- aerosol vaccines 94
- affinity** (antibodies) 33
- affinity maturation** 86–7, 88
- AIDS *see* acquired immunodeficiency syndrome
- allergens 112
- allotypes 86
- alternative pathway** of complement activation 39, 40
- anaphylaxis 112
- antibodies 18–20, 29–38
 - affinity, avidity**, cross-reactivity 33–4
 - see also* affinity maturation
 - binding to antigens 32
 - classes 29
 - and defence against bacteria 48–50
 - functions 35–8
 - neutralising 48–9
 - pathogen binding to receptors 126–7
 - responses 34–5
 - structure 29–32
 - see also* immunoglobulins
- antibody-dependent cell-mediated cytotoxicity (ADCC)** 97
- antibody-mediated immune responses 79–88
- antigen presentation** 19, 23–5
 - by B cells 80–82
 - by macrophages and neutrophils 55–6
 - by non-classical MHC molecules 77–8
 - and mode of immune response 93–4
 - pathways 67
 - role of infection in 78
 - viral interference with 129–30
- antigen-presenting cells (APCs)** 23–4, 55, 56
- antigen processing** 71–2
- antigen receptors** 16–19
- antigenic disguise** 125

antigens 16–17

- T-dependent and T-independent 80, 87
- variation in 123–5

APCs *see* antigen-presenting cells

apoptosis *see* cell death

aspirin 48

autocrine stimulation 75

autoimmune diseases 34, 109, 112, 113

autoimmunity 109, 111–114

avidity (antibodies) 33

B

B cell growth factor *see* interleukins, IL-4

B cell receptors 18–19, 36, 81

B cells 15, 17, 20–21

- antigen presentation by 23, 24, 73

- C3b receptors 42

- lymphomas affecting 110, 111

- role in immune response 79–88

- terminal differentiation 89–90

- see also* memory B cells

bacteria 11

- immune response to 10, 50

BALT *see* bronchus-associated lymphoid tissues

basophils 15, 23, 25, 37

bcl-2 proteins 132

blood platelets 25–6

bone marrow 8, 9

botulism 49

bradykinin 46, 48

bronchus-associated lymphoid tissues (BALT) 8, 13

Burkitt's lymphoma 110, 111

C

C-domains

- of antibody molecule 30, 31

- of T cell receptor 70

capillary permeability 43

carriers of infection 59–60

caspases 98

- inhibitor of caspase-8 132

- CCR5 receptor 115
- CD system (cell surface markers) 16
- CD1 molecules 77–8
- CD3** molecule 18, 72
- CD4** molecule 20, 74, 100
- CD8** molecule 20, 72–3, 100
- CD28 molecule 74–5, 77, 82
- CD40 molecule 82–3, 87
- CD59 molecule 129
- CD64 molecule 36
- CD79 molecule 18
- CD80 molecule 74
- CD86 molecule 74
- CD95 molecule *see* Fas protein
- cell adhesion molecules (CAMs)** 43, 44–5
see also ICAM-1
- cell death 21, 53, 97–9
- cell-mediated immune responses 57, 66–78
- cell migration 23, 25, 42, 43–5, 52
- cell surface markers 14, 18
 nomenclature (CD system) 16
- chemoattractants 25, 26
- chemokines** 21, 43–4, 45
 receptor 115, 122
 viral interference with signalling by 132
- chemotactic molecules 45
- chemotaxis** 23, 25
- cholera 11
- chronic infections, role of macrophages in 59–62
- class I pathway** 67, 71
- class II pathway** 67, 73–4
- class switching** 82–6, 93
- classical pathway** of complement activation 39, 40
- CLIP protein 73
- clonal selection** 17, 89
- cold viruses 94
- colony stimulating factors 21
- complement control proteins 39
- complement system** 37, 39–42, 45
 components produced by macrophages 57–8
 and defence against bacteria 48–50
 pathogen evasion of 127–9
 receptors for activated 52
 and viral defences 94
- corticotropin-releasing factor 48
- costimulatory signals** (macrophages) 55–6, 57, 74–5, 82
- cross-reactivity** (antibodies) 33–4
- CTLA-4 molecule 77, 78
- cytokine-mediated signalling, viral interference 131–2
- cytokines** 20, 21, 122
 cell production and response to 56–7, 90–91
 inflammatory 43, 44–5
 role in sickness 47–8
see also individual cytokine classes
- cytomegalovirus 102–3, 129, 130, 132
- cytotoxic T cells (TC cells)** 15, 19, 67, 72–3
 and viral defences 94, 96–9, 132
- cytotoxicity** 19, 97–9
 cell resistance to 100–1
- D**
- decoy proteins 127, 128
- defensins** 54
- dendritic cells** 15, 25, 73
- diapedesis 44
- diphtheria 49
- disease resistance/susceptibility 115–16, 119, 122–3
- E**
- education** (lymphocytes) 17
- eicosanoids** 46, 48
- endocytosis** 22
- eosinophils** 15, 23
 reactions to helminths 63–6
- eotaxins 64–5
- epitopes** 18–19, 32
- Epstein Barr viruses 110, 111, 130, 132
- exocytosis 64
- external pathway** 67
see also class II pathway
- extracellular infections** 10–11
- F**
- Fab region (of antibody molecule) 30
- Farmer's lung 112
- Fas protein (CD95) 97–8, 99
- Fc receptors** 35–7, 41, 97, 126–7
- Fc region (of antibody molecule) 30, 35
- fever 47
- follicular dendritic cells** 25, 42, 88

formylmethionine (fMet) 45

fungal antigens 112

fungal infections, immune response to 10

G

genes

diversity in humans 93

immunoglobulin-coding 85–6, 87

non-MHC, affecting immune function 122–3

and susceptibility to infections 115–23

germinal centres (lymphoid tissues) 13, 83, 87, 88

glandular fever 110, 132

graft rejection 102–3

granzymes 99

H

Haldane, J. B. S. 115, 117

helminths *see* parasitic worms

helper T cells (Th) 15, 20, 67, 74

TH1, interactions with macrophages 24, 51, 57, 84, 90–92

TH2, interactions with B cells 24, 65–6, 82, 84–8, 90–92

hepatitis viruses 110, 111

hepatitis B 119, 122

herpes viruses 110, 129, 130–31

herpes simplex virus 101, 103, 123, 127, 129, 130

see also Epstein Barr virus

HIV *see* human immunodeficiency virus

HLA-B35 molecule 115, 118

host–pathogen coevolution 7, 132–3

HTLV *see* human T-cell leukaemia viruses

human immunodeficiency virus (HIV) 96, 107–8, 110, 115, 123–4, 129–30

human T-cell leukaemia viruses 110

hypersensitivity 109, 111–114

hypersensitivity reactions 109, 111–13

hypervariable regions

of antibody molecule 30, 32

of T cell receptor 70

I

ICAM-1 molecule 75

IFN γ *see* interferons

immune complexes 35, 41–2, 49, 112

immune deficiency *see* immunodeficiency

immune evasion strategies 123–32

immune response 75, 90–94

non-MHC genes affecting 122–3

phases 10

types 10–11

immune system 7, 8–9

individual variability 93, 120–21

immunization *see* vaccination

immunodeficiency 7, 102–3

and gene mutations 117–18

and tumour development 109

see also human immunodeficiency virus

immunoglobulins 18, 29

class switching 82–6, 93

genes for 85–6

IgA 29, 31, 33, 38, 49–50, 82, 93, 94

receptors for 65

IgD 29

IgE 29, 31, 33, 38, 82, 112

receptors for 36, 37, 65

IgG 29–31, 33, 37, 38, 49, 82–3, 87, 93, 94, 126–7

receptors for 35–6, 52, 65, 97

IgM 29, 31, 33, 37, 79–80

see also antibodies

immunological memory 88–90

immunorepellents 50

immunosuppression 102–3

inducible nitric oxide synthase 54, 56

inflammation 42–5

control 37

and disease symptoms 46–8

mediators 25, 26, 111

processes 46, 47

influenza 123

vaccine 94, 124

integrins 44, 52

interferons 21, 94, 95–6

IFN γ 45, 56–7, 59, 73, 77, 91, 95–6, 131

interleukins 21

IL-1 44–5, 47, 76–7

IL-2 75–7

receptor 75–7

IL-4 83

IL-5 65, 83–4

IL-6 47, 83

IL-10 91

viral analogue 132

IL-12 57, 91

IL-13 83

internal pathway 67

see also class I pathway

intracellular infections 10–11

iron-binding protein *see* lactoferrin

isotypes 86

J

J-chains (of antibody molecule) 31

K

killer inhibitory receptors (KIRs) 94, 130

kinin system 46, 48

Kupffer cells 23

L

lactoferrin 55

Langerhans cells 25

large granular lymphocytes 15, 21–22

see also natural killer cells

lectin pathway of complement activation 39, 40

Leishmania sp. 131

lepomatous leprosy 61, 62, 122

leprosy 61–2

whether genetically determined 119

leukocytes 8–9, 13–14

see also dendritic cells; lymphocytes; phagocytes

leukotriene-B4 52

LFA-1 44, 75

lipo-arabinomannan 78

lipopolysaccharide receptors (macrophages) 56

lymph 13

lymph nodes 8, 13–14, 25, 83, 93

lymphatic system 9, 13–14

lymphocytes 9, 12, 14, 15, 16–22

see also B cells; large granular lymphocytes; T cells

lymphoid tissues 8, 9

see also germinal centres; primary lymphoid tissues;
secondary lymphoid tissues

lymphomas 110, 111

lymphotoxin (TNFb) 100

lysosomes 55, 56

lysozyme 32, 58

lytic pathway of complement 41, 50, 54, 99

M

macrophage activation 51

macrophage-arming factor *see* interferon-d

macrophages 15, 23, 24, 50

functions 51–8

in bacterial infections 58–62

in hypersensitivity reactions 112–13

major basic protein 63

major histocompatibility complex (MHC) 19

see also MHC molecules

malaria

immune responses to 11

pathogen evasion of 125, 127

resistance to 115–16

susceptibility to cerebral 122

vaccine 94

whether genetically determined 119

MALT *see* mucosa-associated lymphoid tissue

mannose receptor 52

markers *see* cell surface markers

mast cells 15, 25, 37

mediators 46, 111, 112

measles virus 131

megakaryocytes 25

membrane attack complexes 50, 54

memory *see* immunological memory

memory cells 89–90

memory B cells 83, 89, 90

memory T cells 89–90

MHC molecules 19, 23

class I 67–70, 96–7, 115–16

class II 67–70

nomenclature for genes 119–20

non-classical 77–8

polymorphisms 117, 119–22

synthesis 57

viral interference with expression of 129–30

monocyte chemotactic proteins 45

monocytes 23, 52

mononuclear phagocytes 9, 15, 23, 73

cytokine production by 45, 51, 57

expression of costimulatory molecules by 74

role in bacterial infections 51, 58–62

role in opsonization 35–6, 41–2

mucosa-associated lymphoid tissues (MALT) 8, 13, 93

mucous membranes, route of infection 11

Mycobacterium leprae 61, 129

Mycobacterium tuberculosis 129, 131

N

naïve (virgin) lymphocytes 17

nasopharyngeal carcinoma 110, 111

natural killer (NK) cells 22, 41

and hypersensitivity reactions 112

and viral defences 22, 41, 96–9, 130

natural selection

in antibody response development 86–7

infectious diseases as agents in 115

Nef protein 129–30

Neisseria gonorrhoeae 12

Neisseria meningitidis 50

neurons, resistance to cytotoxicity 100–1

neutrophils 9, 15, 22–23, 51–2, 54, 55

role in opsonization 35–6, 41–2

nitric oxide 54, 56

O

opsonization 35–7, 41–2, 65

of bacteria 50

P

PALS *see* peri-arteriolar lymphatic sheath

papilloma viruses 110

parasitic worms 11

eosinophil response to 63–6

immune response to 10

pattern recognition (macrophage receptors) 52, 78

perforin 98–9

peri-arteriolar lymphatic sheath (PALS) (of spleen) 14

peroxynitrites 54

Peyer's patches 8, 13, 59

phagocytes 9, 15, 21, 22–3, 51–8

phagocytosis 22, 52–3

phagolysosome 55

phagosome 22, 53

plasma cells 15, 20–21, 24, 79, 83, 89

pleiotropic cytokines 77

polymorphism (genetic variation) 115, 119

in MHC genes 117, 119–22

stable 118

polymorphonuclear leukocytes 15, 22

polymorphs (polymorphonuclear leukocytes) 22

pox viruses 129, 132

primary antibody response 34–5

primary lymphoid tissues 8, 9

prostaglandins 48

proteasome 71

protein A 126

pus 23

R

rabies 11

reactive nitrogen intermediates (RNIs) 53–4

reactive oxygen intermediates (ROIs) 53, 56, 64–5, 129

relative risk of infectious disease 119

S

Salmonella typhi 59

Salmonella typhimurium 59

scavenger receptors 53

schistosomes 122, 125

secondary antibody response 34–5, 89

secondary lymphoid tissues 8, 9, 13–14

selectins 43, 44

skin, defence against infection 10, 11

sleeping sickness *see* trypanosomiasis

spleen 8, 9, 14

stem cells 14, 15

resistance to cytotoxicity 100–1

systemic infections 12

T

T cell receptors 18, 19, 23, 70–71, 72

T cells 15, 17–21

activation and proliferation 75–7

antigen recognition by 18–19, 81–2

CD4⁺ 100

see also cytotoxic T cells (T_C); helper T cells (T_H)

TC cells *see* cytotoxic T cells

T-dependent antigens 80

tetanus 11, 49

TH cells *see* helper T cells

thymus 8
T-independent antigens 80
TNFs *see* tumour necrosis factors
transcription factors 118
transforming growth factors 21
transporter proteins 71
trypanosomiasis 124–5
tuberculoid leprosy 61, 62
tuberculosis 60, 119
tumour necrosis factors (TNFs) 12, 21, 48, 98, 99
 receptor analogues 132
 TNF α 44–5, 57, 59, 122
 TNF β 100
tumours, infection-associated 109–11
twin studies 118–19
typhoid 59–60

V

V-domains
 of antibody molecule 30, 32
 of T cell receptor 70
vaccination 34–5, 93–4, 124
vaccinia virus 132
VDJ recombination 85–6
virgin lymphocytes *see* naïve lymphocytes
viruses 11
 evasion of immune response 126–7, 129–31
 immune defences against 22, 94–102
 immune response to 10
 mutations in 32
Vpu protein 129

W

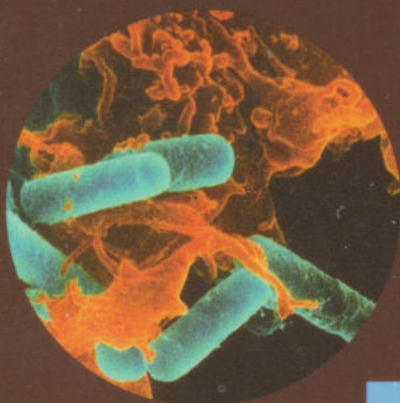
Waldeyer's ring (tonsils and adenoids) 8
white blood cells *see* leukocytes

X

X-linked lymphoproliferative syndrome 110

Y

yellow fever virus 94



S320

INFECTIOUS DISEASE

- 1** PATHOGENS AND PEOPLE
- 2** INFECTIOUS AGENTS
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